

Research Article

Alysiinae (Hymenoptera: Braconidae) parasitoids of the pea leaf miner, *Chromatomyia horticola* (Goureau, 1851) (Diptera: Agromyzidae) in Kermanshah, Iran

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Abstract: *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae) is a highly polyphagous leaf miner that cause severe damage to different crops. Sampling on the Alysiinae (Braconidae) parasitoids of *C. horticola* was performed at various locations of Kermanshah province during 2009–2010. Infested leaves of host plants bearing the larvae and puparia of *C. horticola* were collected and placed inside the plastic rearing boxes and transferred to laboratory. The samples were preserved for 2–4 weeks, until the emergence of parasitoids. Three species of Alysiinae parasitoids were reared and identified in the association with *C. horticola* in Kermanshah including, *Chorebus (Stiphrocera) aphantus* (Marshall, 1986), *Chorebus (Stiphrocera) uliginosus* (Haliday, 1839) and *Dacnusa (Aphanta) hospita* (Foerster, 1862). All three species have been recorded for the first time as parasitoids of *C. horticola*. In addition, *C. aphantus* is newly recorded for the fauna of Iran. An identification key to the parasitoid species is given.

Keywords: Biological control, *Chorebus*, *Dacnusa*, host association.

Introduction

The family Agromyzidae is a large group of the order Diptera, including more than 3,000 worldwide species assembled in about 30 genera (Spencer, 1989). Agromyzids have been considered as one of the most important group of insect pests, especially on vegetables and ornamental plants (Parrella *et al.*, 1984).

Up to now, insecticides were used very frequently to control these pests. However, many researches have recently shown that

the efficiency of chemical insecticides in the control of agromyzids is becoming increasingly less effective because of developing resistance (Parrella *et al.*, 1984; Fergusson, 2004). On the other hand, the widespread use of insecticides causes substantial economic losses from the occurrence of the agromyzids by loss of their natural enemies (LaSalle and Parrella, 1991). Researchers from many countries have attempted to alternate to biological control programs for management of these pests (LaSalle and Parrella 1991; Civelek and LaSalle, 2005; Liu *et al.*, 2009). Parasitoids showed significant effects on suppression of the leaf-miner populations in natural ecosystems and in cultivated areas with

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reduced insecticide use (Johnson *et al.*, 1980).

Accordingly, a complex range of the Hymenoptera species are detected in parasitoid guild of the agromyzid leaf miners that are mainly belong to the superfamily Chalcidoidea (LaSalle and Parrella, 1991) and the family Braconidae (Ichneumonoidea) (Civelek and LaSalle, 2005). Among chalcidoids, many species of eulophids (Civelek and LaSalle, 2005; Gençer, 2009; Fathi, 2011; Mahmoudi *et al.*, 2011) have been recorded as parasitoids of *Chromatomyia horticola* (Goureau) and other agromyzid species, of which several species have successfully been used as biological control agents (Liu *et al.*, 2009). The family Braconidae comprises a diverse assemblage of parasitoids, standing out two subfamilies Alysiinae and Opiinae that include many species associated with agromyzids. Both subfamilies encompass a vast number of species for which very few host records (including Agromyzidae) are evidenced. Recent studies on parasitoids of agromyzids have revealed some associations mainly for Opiinae (Belokobylskij *et al.*, 2004; Civelek and LaSalle, 2005; Çikman *et al.*, 2006). On the other hand, studies on the host association for Alysiinae with agromyzids referring to the works by Griffiths (1966, 1967, 1968a, 1968b, 1984) are recently supplement by several works (Docavo *et al.*, 1987, 2001; Docavo and Tormos, 1988; Tormos and Gayubo, 1990; Tormos *et al.*, 1989, 2008; Pardo *et al.*, 2000, 2001).

Among agromyzid species known from Iran (Dousti, 2010; Shahreki *et al.*, 2012; Hazini *et al.*, 2013), *C. horticola* is a highly polyphagous leaf miner that causes severe damage to different field crops (Fathi, 2011) and in glasshouses (Ostrauskas *et al.*, 2005). This species was recorded on 268 genera of 36 plant families, first of all, Brassicaceae, Fabaceae and Asteraceae (Spencer, 1973). Among the Alysiinae known from Iran, only a single host

association is recorded for *Dacnusa sibirica* Telenga (Fathi, 2011) and nothing for the rest. In the present work, data about occurrence of three Alysiinae species parasitoids of *C. horticola* in Kermanshah province, as well as an identification key to the parasitoid species are given, supplemented with a short diagnosis of each species.

Materials and Methods

Sampling was done in different locations of Kermanshah provinces among 2009–2010. The leaves of host plants infested with the larvae or puparia of the pea leaf miner, *Chromatomyia horticola* were collected and placed inside the plastic rearing boxes (10 cm diameter and 12 cm height). The materials were subsequently divided and cleaned from other insects and mites in laboratory. The rearing boxes were covered with mesh for ventilation and placed in a growth chamber, under constant conditions at the temperature of 25 ± 1 °C, and $65 \pm 5\%$ RH. Samples inside rearing boxes were preserved for 2–4 weeks until the emergence of adult agromyzids and parasitoids. Emerged leaf miners are caught using an aspirator and dropped in the empty test tubes, separately until they naturally died. Adult parasitoids were directly dropped in the tubes containing 75% ethyl alcohol, where they were kept for subsequent pinning or mounting on card. Specimens of *C. horticola* were identified by Dr. Mitsuhiro Sasakawa (Japan). The external morphology of the Alysiinae parasitoids was studied and illustrated using Leica™ S8 APO stereomicroscope equipped with a Nikon™ D700 digital camera. Alysiinae specimens were identified following Tobias (1986) key. For the terminology of the morphological features and sculptures, measurements and wing venation nomenclature see Sharkey and Wharton (1997). General distribution data for each species is provided according to Yu *et al.* (2012). All specimens were deposited in the Naturhistorisches Museum Wien (Vienna, Austria; NHMW).

Results

Three species of Alysiinae parasitoids were reared and identified in the association with *C. horticola* in Kermanshah: *Chorebus (Stiphrocera) aphantus* (Marshall, 1986); *Chorebus (Stiphrocera) uliginosus* (Haliday, 1839) and *Dacnusa (Aphanta) hospita* (Foerster, 1862). *Chorebus aphantus* is recorded as new for the fauna of Iran. The key for the identification of these three species is given below.

Key to the Alysiinae parasitoids of *Chromatomyia horticola* in Kermanshah

1. Pubescence on metapleuron sparse, or if dense, then distributed evenly and directed downwards to hind coxa (Figs 5B, 5D). Mandible with 3 teeth (Fig. 5A). Vein RS + M absent, so first discal and first submarginal cells confluent (Fig. 5F). First metasomal tergite 1.3–1.5 times as long as its apical width (Fig. 5D). Antenna 18-segmented (Fig. 5E).
.....***Dacnusa hospita* (Foerster)**
- Pubescence on metapleuron usually in form of dense rosette of radiating setae around a raised swelling (Figs 1C, 3C). Mandible with 4 teeth (Figs. 1D, 2A, 3D, 4A). Vein RS + M present (Figs. 1F, 3F). First metasomal tergite 1.9–2.1 times as long as its apical width (Fig. 2C, 4C). Antenna 24–38-segmented (Figs. 1E, 3E).
..... **(Genus: *Chorebus* Haliday) 2**
2. Hind coxae without tuft of setae (Fig 2D). Pronotum lacking dense pubescence. Occiput with sparse setae (Fig. 2B). Head in dorsal view twice as wide as its median length. Hind femur 5.0 times as long as its maximum width (Fig. 2D). Ovipositor shorter than metasoma (Fig 1A).....***Chorebus aphantus* (Marshall)**
- Hind coxae with tuft of setae (Fig. 3C). Pronotum densely pubescent. Occiput pubescent (Fig. 4B). Head in dorsal view 1.6 times as wide as its median length. Hind femur

3.6–3.7 times as long as its maximum width (Fig. 4D). Ovipositor as long as metasoma (Fig. 3A).....***Chorebus uliginosus* (Haliday)**

***Chorebus (Stiphrocera) aphantus* (Marshall, 1896) (Figs. 1, 2)**

Materials examined: 1♀, 1♂, Iran, Kermanshah province, Bistoon (code 08), 07-June-2009, reared from *Chromatomyia horticola* on *Malva neglecta* Wallr., Leg.: F. Hazini.

Main characters of the species (female). Body length 1.6–2.1 mm. Head in dorsal view 2.0 times as wide as its median length (Fig. 2B) and 1.5 times as wide as mesoscutum. Face 1.4 times as wide as high (Fig. 2A). Mandibles 4-dentate (Figs. 1D, 2A), 1.4 times as long as wide. Occiput with sparse setae (Fig. 2B). Antenna 22–31-segmented (Fig. 1E). First flagellar segment 3.7 times as long as its apical width. Mesosoma in lateral view 1.4 times as long as height (Fig. 1C). Mesoscutum 1.15 times as long as its maximum width (Fig. 2B). Mesoscutal pit present, oval. Pronotum lacking dense pubescence. Precoxal suture present, reaching anterior margin of and not reaching posterior margin of mesopleuron. (Fig. 1C). Posterior mesopleural furrow smooth. Propodeum sculptured, densely pubescent (Fig. 2C). Hind coxae without tuft of setae. Hind femur 5.0 times as long as its maximum width (Fig. 2D). First metasomal tergite 1.9 times as long as its apical width, almost smooth (Fig. 2C). Ovipositor shorter than metasoma (Fig. 1A, 2D). Main colour brown to dark brown; legs yellow (Figs. 1A and 1B).

General distribution: Palaearctic (Austria, Azerbaijan, China, Denmark, Germany, Hungary, Iceland, Iran (**new record**), Ireland, Poland, Russia, Spain, Sweden, Switzerland, Turkey, United Kingdom).

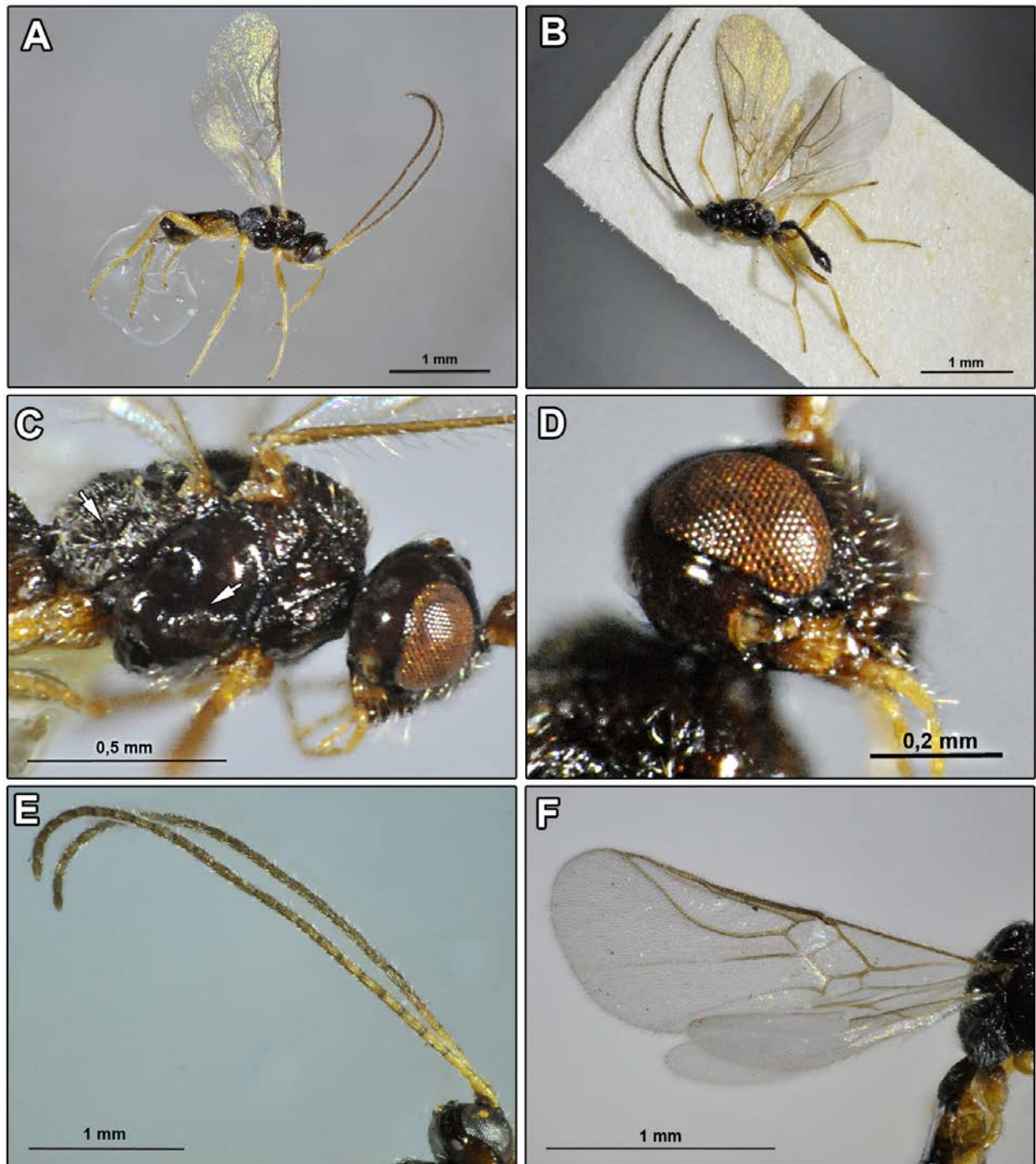


Figure 1 *Chorebus aphantus* (Marshall, 1896): A. Lateral habitus of female, B. lateral habitus of male, C. Lateral aspect of head and mesosoma, D. Fronto-lateral aspect of head, E. Antennae, F. Fore wing.

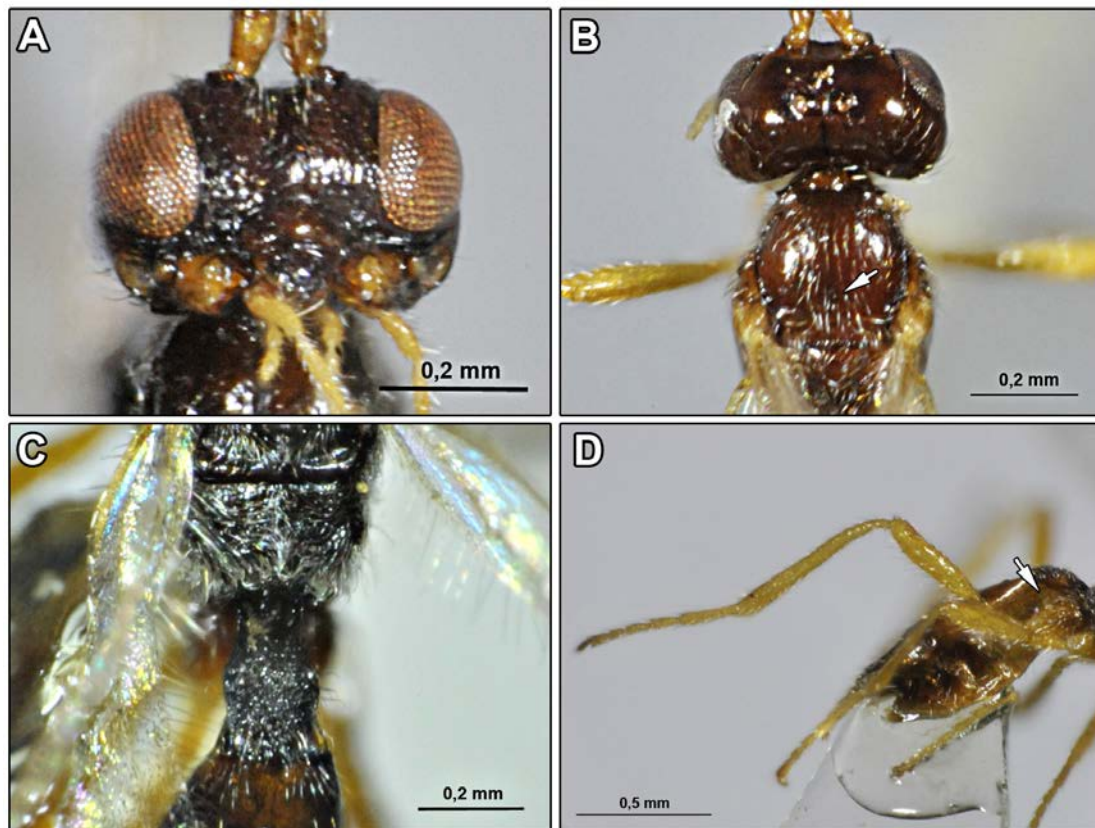


Figure 2 *Chorebus aphantus* (Marshall, 1896): A. Face and mouth parts, B. Dorsal aspect of head and mesosoma, C. Dorsal aspect of propodeum and first metasomal tergite, D. Lateral aspect of metasoma and hind legs.

***Chorebus (Stiphrocera) uliginosus* (Haliday, 1839) (Figs. 3, 4)**

Materials examined: 1♂, Iran, Kermanshah province, Kermanshah (code 48), 18-April-2009, reared from *C. horticola* on *Trifolium repens* L., Leg.: F. Hazini; 2♀, Kermanshah province, Sarab-Niloufar (code 62), 01-May-2009, reared from *C. horticola* on *Lactuca orientalis* (Boissier), Leg.: F. Hazini.

Main characters of the species (Female). Body length 1.9–2.1 mm. Head in dorsal view 1.6 times as wide as its median length (Fig. 4B) and 1.3 times as wide as mesoscutum. Face 1.4 times as wide as high (Figs. 4A). Mandibles 4-dentate (Figs. 3D, 4A), 1.4 times as long as wide. Occiput densely pubescent (Fig. 4B). Antenna 22–38-segmented (Fig. 3E). First flagellar segment 3.3 times as long as its apical width. Mesosoma in lateral view 1.5 times as long

as height (Fig. 3C). Mesoscutum as long as its maximum width (Fig. 4B). Mesoscutal pit present, oval. Pronotum densely pubescent. Precoxal suture present, reaching anterior margin of and not reaching posterior margin of mesopleuron (Fig. 3C). Posterior mesopleural furrow smooth. Propodeum sculptured, densely pubescent (Fig. 4C). Hind coxae with tuft of setae (Fig. 3C). Hind femur 3.6–3.7 times as long as its maximum width (Fig. 4D). First metasomal tergite 2.1 times as long as its apical width, pubescent (Fig. 4C). Ovipositor as long as metasoma (Figs. 3A, 4D). Main colour brown and dark brown; legs brown yellowish (Figs. 3A, 3B). **General distribution:** Palearctic (Belgium, Germany, Hungary, Iran, Ireland, Italy, Korea, Lithuania, Mongolia, Netherlands, Poland, Romania, Russia, Sweden, Ukraine, United Kingdom).

***Dacnusa (Aphanta) hospita* (Foerster, 1862) (Fig. 5)**

Materials examined: 1♀ 1♂, Iran, Kermanshah province, Kuzaran (34°50'99.52"N;46°59'82.82"E) (code 64), 01-May-2009, reared from *C. horticola* on *Matthiola* sp., Leg.: F. Hazini.

Main characters of the species (Female). Body length 1.7–1.8 mm. Head in dorsal view 2.0 times as wide as its median length (Fig. 5C) and 1.4 times as wide as mesoscutum. Face 1.6 times as wide as high (Fig. 5A). Mandible 3-dentate (Fig. 5A), 1.1 times as long as wide. Occiput with sparse setae. Antenna 18-segmented (Fig. 5E). First flagellar segment 4.5 times as long as its apical width. Mesosoma in lateral view (Fig. 5B) 1.3 times as long as height. Mesoscutum as

long as its maximum width (Fig. 5C). Mesoscutal pit present, oval. Pronotum lacking dense pubescence. Precoxal suture present, reaching anterior margin of and not reaching posterior margin of mesopleuron (Fig. 5C). Posterior mesopleural furrow smooth. Propodeum smooth, with sparse setae (Fig. 5D). Hind femur 4.5 times as long as its maximum width (Fig. 5B). First metasomal tergite 1.3–1.5 times as long as its apical width (Fig. 5D). Ovipositor shorter than metasoma. Main colour brown and dark brown; legs brown yellowish (Fig. 5B).

General distribution: Palearctic (Bulgaria, China, Denmark, Germany, Hungary, Iran, Ireland, Italy, Spain, United Kingdom).

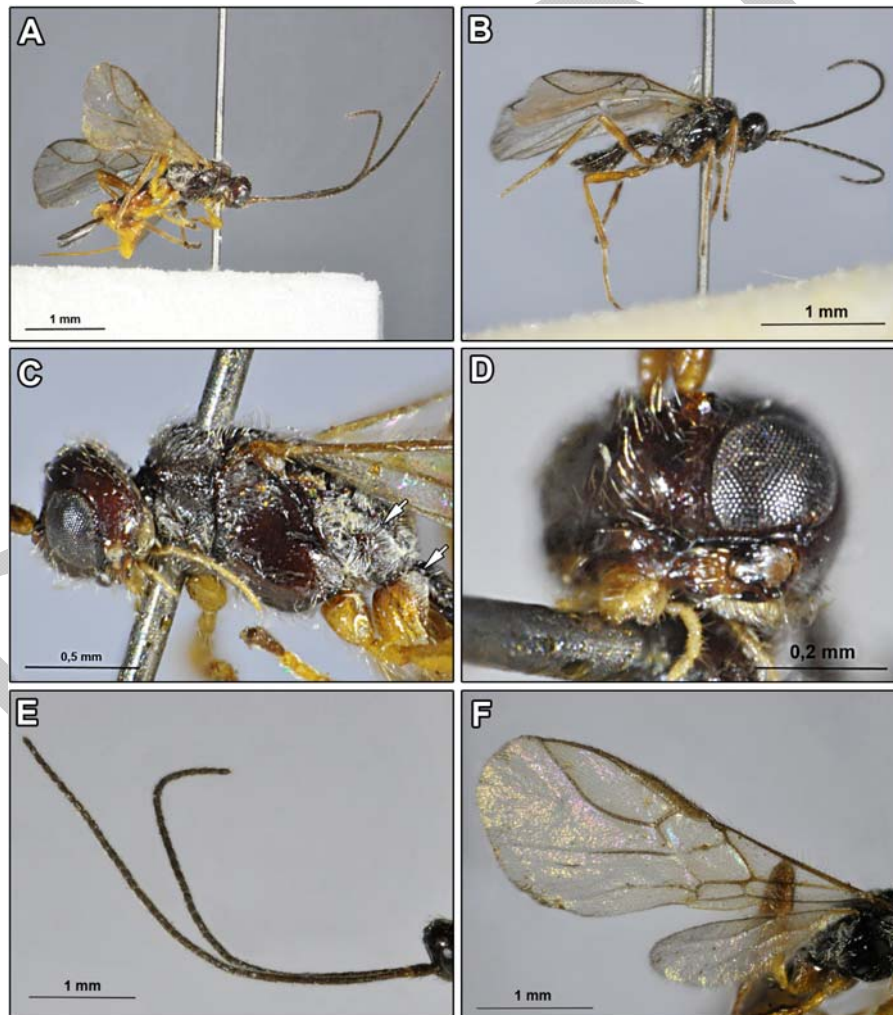


Figure 3 *Chorebus uliginosus* (Haliday, 1839). A. Lateral habitus of female, B. lateral habitus of male, C. Lateral aspect of head and mesosoma, D. Fronto-lateral aspect of head, E. Antennae, F. Fore wing.

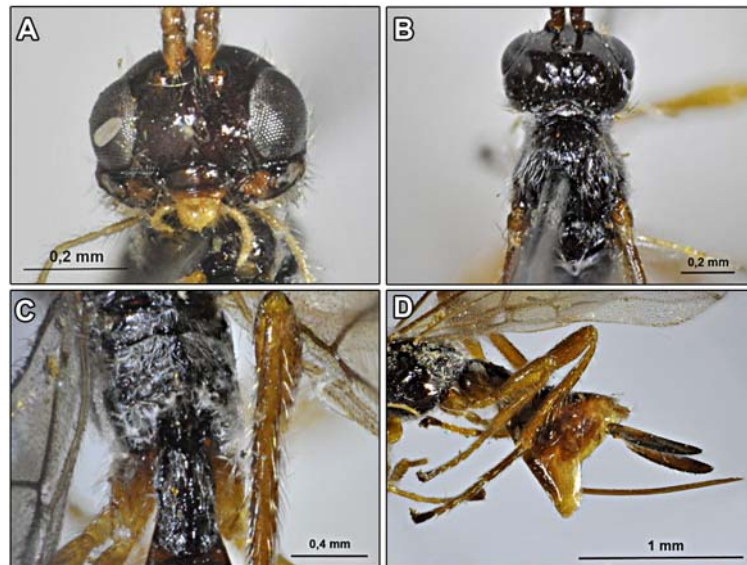


Figure 4 *Chorebus uliginosus* (Haliday, 1839): A. Face and mouth parts, B. Dorsal aspect of head and mesosoma, C. Dorsal aspect of propodeum and first metasomal tergite, D. Lateral aspect of metasoma and hind legs.

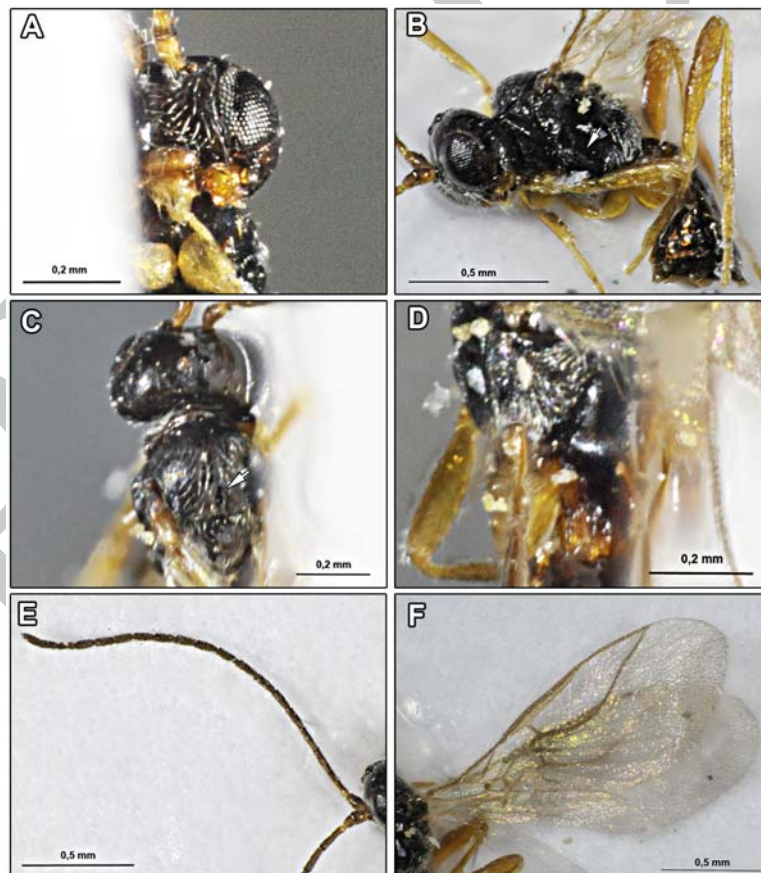


Figure 5 *Dacnusa hospita* (Foerster, 1862): A. Face and mouth parts, B. Lateral habitus of female, C. Dorsal aspect of head and mesosoma, D. Dorsal aspect of propodeum and first metasomal tergite, E. Antennae, F. Fore wing.

Discussion

Chromatomyia horticola is widely distributed in many countries in associations with numerous host plant species (Spencer, 1973). This species has been recorded from eastern (Shahreki *et al.*, 2012), central (Dousti, 2010) and western parts (Fathi, 2011; Hazini *et al.*, 2013) of Iran, and most probably distributed in the whole country. While many eulophids (Chalcidoidea) have been recorded as parasitoids of the agromyzid leaf miners from Iran (Asadi *et al.*, 2006; Zahiri *et al.*, 2004; Dousti *et al.*, 2008), the Alysiinae species (as well as Opiinae) has generally been ignored in previous studies. However, checking with neighboring countries, many studies were carried out in Turkey with parasitoid guild of the agromyzids (Civelek and LaSalle, 2005; Çikman *et al.*, 2006; Gençer, 2009) revealing many associations of the eulophids as well as braconids (Braconinae, Microgastinae, Opiinae), but excluding Alysiinae.

In general, there is only a single host record among the known Alysiinae of Iran (Khajeh *et al.*,

2014) referring to the association of *D. sibirica* with *C. horticola* (Fathi, 2011). Among the collected parasitoids, only few host records are available for *Chorebus aphantus* including *Chromatomyia milii* (Kaltenbach) (Michalska, 1973) *Chromatomyia nigra* (Meigen) (Griffiths, 1967) and *Liriomyza flaveola* (Fallen) (Griffiths, 1968a). On the other hand, *Chorebus uliginosus* has been recorded in association with dipterans of the family Ephydriidae, *Hydrellia griseola* (Fallen) (Yu *et al.*, 2012), *Hydrellia nigripes* (Zetterstedt) (Yu *et al.*, 2012), as well as Agromyzidae, *Liriomyza strigata* (Meigen) (Hedwig, 1955). *Chromatomyia horticola* is a new host record for both *Chorebus* species. *Dacnusa hospita* was also recorded in association with few species of Agromyzidae excluding *C. horticola* (Griffiths, 1968a; Docavo and Tormos, 1988). Many other Alysiinae species belong to genera *Chorebus*, *Dacnusa* and *Dapsilarthra* Foerster have been reported as parasitoids of *C. horticola* (Table 1) in different countries.

Table 1 World list of Alysiinae parasitoids of *Chromatomyia horticola*.

Parasitoid species	Country	Reference	Occurrence in Iran
<i>Chorebus aphantus</i> (Marshall, 1896)	Iran	Present study	+
<i>Chorebus denticurvatus</i> Pardos, Tormos and Verdu, 2001	Spain	Prado <i>et al.</i> (2001)	-
<i>Chorebus flavipes</i> (Goureaux, 1851)	Spain	Docavo <i>et al.</i> (1987)	+
<i>Chorebus longiventris</i> Docavo, Fischer and Tormos, 2001	Spain	Docavo <i>et al.</i> (2001)	-
<i>Chorebus misellus</i> (Marshall, 1895)	Spain	Tormos <i>et al.</i> (1989)	-
<i>Chorebus nanus</i> (Nixon, 1943)	Germany	Griffiths (1984)	-
<i>Chorebus petiobrevis</i> Docavo, Fischer and Tormos, 2001	Spain	Docavo <i>et al.</i> (2001)	-
<i>Chorebus sativi</i> (Nixon, 1943)	Spain	Tormos and Gayubo (1990)	-
<i>Chorebus uliginosus</i> (Haliday, 1839)	Iran	Present study	+
<i>Dacnusa areolaris</i> (Nees, 1811)	Spain, Italy	Tormos <i>et al.</i> (1989), Priore and Tremblay (1995)	-
<i>Dacnusa hospita</i> (Foerster, 1862)	Iran	Present study	+
<i>Dacnusa laevipectus</i> Thomson, 1895	Spain	Docavo and Tormos (1988)	-
<i>Dacnusa maculipes</i> Thomson, 1895	undefined	Griffiths (1966)	-
<i>Dacnusa nipponica</i> Takada, 1977	Japan	Takada (1977)	-
<i>Dacnusa rodriguezi</i> Docavo and Tormos, 1997	Spain	Pardo <i>et al.</i> (2000)	-
<i>Dacnusa sasakawai</i> Takada, 1977	Japan	Takada (1977)	-
<i>Dacnusa sibirica</i> Telenga, 1935	Spain, Iran	Tormos and Gayubo (1990), Fathi (2011)	+
<i>Dapsilarthra rufiventris</i> (Nees, 1812)	undefined	Griffiths (1966)	-

Knowledge of the parasitoids of Agromyzidae has increasingly become important in biological control programs. Despite the Alysiinae are an important group of the parasitoids, even the faunal studies have recently started in Iran (Khajeh *et al.*, 2014) and almost nothing is available about their biology and host associations. In this research, we found valuable data on Alysiinae parasitoids of an important leaf miner, *C. horticola*, while the host range pattern of parasitoids also needs to be determined. Furthermore, additional studies both on their frequency and seasonal dynamics as well as on their host specificity are also required to provide the background for applicability of these parasitoids in further biological control programs.

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زنبورهای آلزینه پارازیتوئید مگس مینوز نخودفرنگی، *Chromatomyia horticola* (Goureau, 1851) (Diptera: Agromyzidae) در کرمانشاه، ایران

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چکیده: مگس مینوز نخودفرنگی، *Chromatomyia horticola* (Goureau, 1851) گونه‌ای از مینوزها با دامنه میزبانی بسیار وسیع است که آسیب زیادی به محصولات مختلف وارد می‌کند. نمونه‌برداری از زنبورهای پارازیتوئید این گونه از زیرخانواده Alysiinae (Braconidae) در مناطق مختلف استان کرمانشاه طی سال‌های ۱۳۸۸-۱۳۸۹ انجام شد. برگ‌های گیاهان میزبان حاوی لارو و شفیره مگس مینوز از طبیعت جمع‌آوری و داخل ظروف پلاستیکی ویژه نگهداری حشرات قرار داده شدند. نمونه‌ها به مدت ۲ تا ۴ هفته در داخل ظروف تا زمان خروج حشرات کامل مگس مینوز و پارازیتوئیدهای آن نگهداری شدند. مگس‌های خارج شده توسط اسپیراتور از درون ظروف، جمع‌آوری و به‌طور جداگانه داخل لوله آزمایش خالی ریخته شدند تا پس از مرگ طبیعی به‌صورت خشک باقی بمانند. زنبورهای پارازیتوئید مستقیماً داخل ظروف حاوی الکل ۷۵ درصد ریخته شده و در نهایت به‌منظور مطالعه خصوصیات مرفولوژیک به روش دقیق سوزن زده شده و یا روی پلاک قرار گرفتند. از بین نمونه‌های پارازیتوئید جمع‌آوری شده، سه گونه متعلق به زیرخانواده Alysiinae شامل *Chorebus (Stiphrocera) aphantus* (Marshall, 1986)، *Chorebus (Stiphrocera) uliginosus* (Haliday, 1839) و *Dacnusa (Aphantu) hospita* (Foerster, 1862) شناسایی شدند. ارتباط هر سه گونه به‌عنوان پارازیتوئید مگس *C. horticola* برای اولین بار ثبت می‌شود. همچنین گونه *Chorebus aphantus* برای اولین بار از ایران گزارش می‌شود. کلید شناسایی گونه‌های جمع‌آوری شده نیز ارائه شده است.

واژگان کلیدی: کنترل زیستی، *Chorebus*، *Dacnusa*، روابط میزبانی