



Fauna of the genus *Diglyphus* (Hymenoptera: Eulophidae) in the alfalfa fields of Iran

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Abstract. Considering the importance of the species of the genus *Diglyphus* Walker, 1844 (Hym., Eulophidae: Eulophinae) in the biological control of leafminers, a faunal study was conducted during 2018–2020 in alfalfa fields in West Azarbaijan province, northwestern Iran. Six *Diglyphus* species were collected and identified, i.e. *Diglyphus crassinervis* Erdős, 1958; *D. isaea* (Walker, 1838); *D. poppoea* Walker, 1848; *D. propodealis* Szelenyi, 1978; *D. pusztensis* (Erdős & Novicky, 1951); and *D. sabulosus* Erdős, 1951; of which *D. propodealis* is a new record for Iranian fauna, that is known just from its type locality, Hungary. Notes on diagnostic characters and illustrations of *D. propodealis* are given. Its association with alfalfa fields is the first association report. All six reported species are new to the West Azarbaijan province. A distribution map of reported species is provided and the known *Diglyphus* species in association with alfalfa in Iran are also reviewed.

Keywords: Biological control, *Diglyphus propodealis*, Eulophinae, *Medicago sativa*, parasitoid

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Introduction

Alfalfa or Lucerne, *Medicago sativa* L. (Fabaceae), is one of the most economically important perennial crops. Alfalfa has a rich protein content and grows worldwide as one of the major forage crops which is used as livestock feed. Furthermore, its cultivation enriches the soil with biological nitrogen and improves soil fertility (Bolton, 1962; Hirsh, 2014). The alfalfa cultivation area in Iran is ca. 502759 ha and West Azarbaijan province has the second ranking (SCI, 2018). In the guild of leaf feeders of alfalfa, leafminer larvae generally feed on the leaf parenchyma (Spencer, 1973; Noyes, 2001). The main alfalfa leafminers of economic importance are *Agromyza frontella* (Rondani, 1875); *A. nana* Meigen, 1830; *Liriomyza congesta* (Becker, 1903); *L. sativae* Blanchard, 1938; and *L. trifoliarum* Spencer, 1973; which all belong to the family Agromyzidae (Spencer, 1973). In the biological control of leafminers, species of the genus *Diglyphus* Walker, 1844 (Hym., Eulophidae) are well-known parasitoids of the leafminers (Gordh & Hendrickson, 1979; Murphy & LaSalle, 1999; Noyes, 2001; Lotfalizadeh *et al.*, 2015), keeping the population of agromyzid leafminers (Dip., Agromyzidae) below the economic threshold (Bouček & Askew, 1968; Spencer, 1973; Noyes, 2019). *Diglyphus begini* and *D. isaea* are practically used in greenhouses and farms to control agromyzid leafminers (Gordh & Hendrickson, 1979; Heinz & Parrella, 1989; Minkenberg & Parrella, 1990). In alfalfa fields, *A. frontella* is parasitized by four *Diglyphus* species including *D. begini* (Hutchinson *et al.*, 1997), *D. isaea* (Drea *et al.*, 1982), *D. intermedius* and *D. pulchripes* (Hendrickson & Barth, 1979a). According to Drea *et al.*, 1982, *A. nana* is parasitized by *D. isaea*. Also *L. congesta* is parasitized by *D. isaea* (Drea *et al.*, 1982) and *D. minoens* (Cikman, 2012); and *L. trifoliarum* is parasitized by *D. intermedius* (Hendrickson, 1979). Species

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of the genus *Diglyphus* are parasitoids with a cosmopolitan distribution including 41 known species, worldwide, of which 31 species are distributed in the Palaearctic region (Noyes, 2019), and 10 species in Iran (Hesami *et al.*, 2018). In addition to the published literatures on the identification of species of this genus in the world, an identification key for Iranian species has already been published by Yefremova *et al.* (2007). Identifying parasitoid species associated with important crops will provide the information needed to make the right management decisions to deal with serious pests of those crops (Edde, 2021). Considering this issue and the importance of alfalfa as a strategic product, the aims of the present study are to (1) identify the *Diglyphus* fauna in the alfalfa fields of Iran, (2) review the fauna of *Diglyphus* species in alfalfa fields, and (3) report a new species for Iranian fauna.

Materials and methods

Sampling was carried out on alfalfa fields between 2018 and 2020 in different parts of West Azarbaijan Province. A sweep net was used to collect samples from the fields. Then, the parasitoid specimens were collected from the net using an aspirator and transferred to 75% alcohol. Specimens were examined for detecting the genus *Diglyphus* using a ZEISS-Stemi SV8 stereomicroscope, and identification keys provided by Gibson *et al.* (1997) and Burks (2003). Morphological terminology followed Gibson (1997) and Yoder *et al.* (2010). To preserve the specimens, the proposed method of Noyes (1982) was used so that the specimens were dehydrated. The CPD method was used to dry the specimens, and an acetic acid bath was used to relax the specimens. The morphological characteristics of *Diglyphus* species were studied using an Olympus SZH stereomicroscope. To identify the specimens at the species level, the identification key provided by Hansson & Navone (2017) was used. Identified specimens were deposited in the Plant Protection Department of Urmia University (PPDUU).

Results

In total, 44 *Diglyphus* specimens were collected from alfalfa fields and identified as six species, of which *Diglyphus propodealis* is a new record for the Iranian fauna. In addition, the association of *D. propodealis* with alfalfa fields was documented for the first time.

Taxonomy

Order Hymenoptera

Superfamily Chalcidoidea

Family Eulophidae

Genus *Diglyphus* Walker, 1844

Diglyphus crassinervis Erdős, 1958

Material examined. IRAN • 1 ♂ (PPDUU): West Azarbaijan province, Urmia; 37°26'48.365" N, 45°2'27.373" E; 1894 m a.s.l.; August 13, 2018; M. Jafarlu leg.

Remarks. *Diglyphus crassinervis* is an ectoparasitoid of several species of leafminers belonging to the Agromyzidae family. This species is generally distributed in the Palaearctic region (Noyes, 2019), and has been reported in Isfahan (Ghahari & Yefremova, 2013) and Tehran (Asadi *et al.*, 2006) provinces in Iran (Fig. 2).

Diglyphus isaea (Walker, 1838)

Material examined. IRAN-West Azarbaijan province • 4 ♀♀ 3 ♂♂ (PPDUU): Khoy; 38°39'28.426" N, 44°39'27.356" E; 2013 m a.s.l.; July 18, 2019; M. Jafarlu leg. • 2 ♀♀ 2 ♂♂ (PPDUU): Salmas; 38°11'31.077" N, 44°43'38.631" E; 1411 m a.s.l.; September 9, 2020; *ibid.* • 1 ♀ (PPDUU): Sardasht; 36°8'2.836" N, 45°28'10.634" E; 1587 m a.s.l.; October 21, 2019; *ibid.* • 2 ♀♀ (PPDUU): Takab; 36°23'37.729" N, 47°4'6.663" E; 1872 m a.s.l.; October 28, 2020; *ibid.* • 3 ♀♀ 2 ♂♂ (PPDUU): Urmia; 37°26'48.365" N, 45°2'27.373" E; 1894 m a.s.l.; August 13, 2018; *ibid.*

Remarks. *Diglyphus isaea* is an ectoparasitoid of several species of leafminers in the dipterous families Agromyzidae and Tephritidae; lepidopterous families Gracillariidae, Lyonetiidae and Nepticulidae. This species has a cosmopolitan distribution and has been reported from all ecozones except the Antarctic and Oceania (Noyes, 2019). In Iran, it has been reported from the provinces of Chaharmahal and Bakhtiari (Yefremova *et al.*, 2007), Fars (Dousti *et al.*, 2008), Isfahan, and Mazandaran (Ghahari & Yefremova, 2013), Sistan and Baluchestan (Shahreki *et al.*, 2012), Tehran (Asadi *et al.*, 2006), and especially in East Azarbaijan province (Lotfalizadeh *et al.*, 2015) (Fig. 2), which has been reared on the dipterous leafminers, i.e. *Liriomyza trifolii* (Burgess, 1880) and *Chromatomyia horticola* (Goureau, 1851) belong to the Agromyzidae family and

lepidopterous leafminer, *Phyllonorycter medicaginella* (Gerasimov, 1930) from the Gracillariidae family on alfalfa.

***Diglyphus poppoea* Walker, 1848**

Material examined. IRAN-West Azarbaijan province • 2 ♀♀ (PPDUU): Chaldoran; 39°2'44.997" N, 44°20'47.022" E; 1881 m a.s.l.; August 26, 2019; M. Jafarlu leg. • 3 ♀♀ (PPDUU): Maku; 39°18'29.571" N, 44°20'4.007" E; 1962 m a.s.l.; *ibid.*

Remarks. *Diglyphus poppoea* is an ectoparasitoid of several species of leafminers belonging to the Agromyzidae family. This species is distributed in the Palaearctic region (Noyes, 2019), and in Iran, it has been reported in the provinces of Fars (Dousti *et al.*, 2008), Golestan, and Isfahan (Ghahari & Yefremova, 2013), Sistan and Baluchestan (Shahreki *et al.*, 2012) (Fig. 2).

***Diglyphus propodealis* Szelényi, 1978 (Figs 1, 2)**

Material examined. IRAN-West Azarbaijan province • 2 ♀♀ (PPDUU): Bokan; 36°28'38.604" N, 46°8'50.813" E; 1347 m a.s.l.; September 9, 2020; M. Jafarlu leg. • 2 ♀♀, 1 ♂ (PPDUU): Khoy; 38°29'59.997" N, 44°33'36.717" E; 2173 m a.s.l.; July 18, 2019; *ibid.*

Diagnosis. Female. Body color metallic-green with some pale markings on legs (Fig. 1A). Scape completely dark (Figs 1A, C). Femora dark with a quarter to one-fifth end yellow or white; fore tibiae dark with apical one-third bronzy; mid and hind tibiae dark with apical one-fifth yellow or white (Fig. 1A). Wings hyaline and covered with dense setae; fore wing speculum with a few scattered setae; stigmal vein 2.6 × as long as wide; marginal vein 4 × as long as stigmal vein (Figs 1A, D). Propodeum densely reticulate (Figs 1E, F).

Male. Similar to female except: slimmer scape. Femora dark with apical two-fifths yellow or white; all tibiae at apical one-third yellow or white. Marginal vein 3 × as long as stigmal vein (Fig. 1B).

Remarks. *Diglyphus propodealis* is a Palaearctic species (Szelényi, 1978) and so far, its primary host and plant association has not been identified. In addition, comparing the examined specimens with the published description of the holotype (Szelényi, 1978; Hansson & Navone, 2017) reveals differences in color patterns, which have been discussed in the discussion section.

***Diglyphus puztensis* (Erdős & Novicky, 1951)**

Material examined. IRAN • 3 ♂♂ (PPDUU): West Azarbaijan province, Mahabad; 36°44'5.584" N, 45°37'26.044" E; 1360 m a.s.l.; September 9, 2020; M. Jafarlu leg.

Remarks. *Diglyphus puztensis* is an ectoparasitoid of several species of leafminers belonging to the Agromyzidae family (Noyes, 2019). This species is distributed in the Palaearctic region (Noyes, 2019), and in Iran, it has been reported in Fars province (Hesami *et al.*, 2010) (Fig. 2).

***Diglyphus sabulosus* Erdős, 1951**

Material examined. IRAN-West Azarbaijan province • 3 ♀♀, 5 ♂♂ (PPDUU): Khoy; 38°39'28.426" N, 44°39'27.356" E; 2013 m a.s.l.; July 18, 2019; M. Jafarlu leg. • 3 ♂♂ (PPDUU): Miandoab; 36°55'25.352" N, 46°3'20.112" E; 1291 m a.s.l.; September 3, 2018; *ibid.*

Remarks. *Diglyphus sabulosus* is an ectoparasitoid of *Liriomyza* sp. (Dip., Agromyzidae) (Herting, 1978; Yefremova *et al.*, 2011; Yefremova, 2015). This species is distributed in the Palaearctic region (Noyes, 2019) and has been reported in Qazvin and Tehran provinces in Iran (Farahbakhsh, 1961; Davatchi & Shojaei, 1989) (Fig. 2).

Discussion

The present study is the first review of *Diglyphus* species associated with alfalfa fields. There were sporadic reports of the presence of some species of *Diglyphus* on alfalfa (Table 1). Among the species identified in this study, *D. isaea* was previously reported on alfalfa by other researchers (Table 1). Hereby, five species related to alfalfa fields are also reported (Table 1). It is important to mention that these species were not obtained through rearing, but were collected exclusively by sweep net in the alfalfa fields.

Among the six species collected from alfalfa fields in this research, no plant association has previously been reported for *D. propodealis* (Szelényi, 1978; Hansson & Navone, 2017; Noyes, 2019). *Diglyphus isaea* has been collected from 20 families of plants (Noyes, 2019), including alfalfa (Gordh & Hendrickson, 1979; Hendrickson & Barth, 1979b; Drea *et al.*, 1982; Hansson, 1987; Askew *et al.*, 2001; Lotfalizadeh *et al.*, 2015). So far, *D. puztensis* has been collected from the plants of the families Brassicaceae (Gençer, 2009), Solanaceae (Nishino & Uchida, 1999), and Poaceae (Hesami *et al.*, 2010). Three other species identified in the current study, including *D. crassinervis*, *D. poppoea*, and *D. sabulosus* have been previously collected from plants of the Fabaceae family, but so far, haven't been reported from alfalfa (Noyes, 2019). *Diglyphus propodealis*, which is new to the Iranian fauna, had previously been reported only from Hungary (Szelényi, 1978) and this is the second report of this species in the world. The association of *D. propodealis* with alfalfa

fields may suggest that the wasp is a parasitoid of leafminer(s) on alfalfa, which requires further investigation to examine this conjecture. In the present study, the holotype of *D. propodealis* was not examined and comparisons were made using published literatures (Szelényi, 1978; Hansson & Navone, 2017). So, some differences in color patterns are observed in *D. propodealis* in this study compared to its holotype. As in the holotype, femora in both sexes are yellow or white at apical one-fourth to one-fifth (Szelényi, 1978; Hansson & Navone, 2017), but here the male femora are yellow or white at apical two-fifths (Fig. 1B). Also in the holotype, the fore tibia is completely dark and the mid and hind tibiae are dark at basal four-fifths and yellow or white at apical one-fifth (Szelényi, 1978; Hansson & Navone, 2017), while in our examined specimens, the fore tibia in female is bronzy at apical one-third (Fig. 1A). The mid and hind tibiae in female are similar to the holotype, but in male all tibiae are yellow or white at apical one-third (Fig. 1B). Including a new record for the Iranian fauna in this study, so far 11 species of the genus *Diglyphus* have been reported from Iran (Hesami *et al.*, 2018). Among the collected species, *D. isaea* was the most frequent species. Generally, this species is distributed in most parts of the country (Asadi *et al.*, 2006; Yefremova *et al.*, 2007; Dousti *et al.*, 2008; Shahreki *et al.*, 2012; Ghahari & Yefremova, 2013; Lotfalizadeh *et al.*, 2015) (Fig. 2). So, *D. isaea* probably has the highest rate of parasitism among species of the genus, which is to be expected, as seen in the findings of other researchers (Cikman, 2012; Lotfalizadeh *et al.*, 2015; Muchemi *et al.*, 2018). *Liriomyza sativae* Blanchard, 1938 is one of the economically important leafminers on alfalfa (Spencer, 1973) and so far, eight species of the genus *Diglyphus* have been reported as parasitoids of this pest. During the study of the genus *Diglyphus* in Turkey, four species (*D. chabrias*; *D. crassinervis*; *D. minoews*; and *D. sensilis*) were reported as parasitoids of *L. sativae* (Yefremova *et al.*, 2011). In addition, in other studies, *D. begini* (Zehnder & Trumble, 1984); *D. intermedius* (Burks, 1967); *D. isaea* (Bouček, 1988); and *D. wani* (Ye *et al.*, 2018) were also detected as parasitoids of the latter leafminer. But in none of the cases mentioned, the associated plant wasn't alfalfa. Among these eight species of *Diglyphus*, five species are related to alfalfa (Table 1). Therefore, it is possible that some of them will be parasitoids of *L. sativae* on alfalfa. Examining this hypothesis and finding the first species of *Diglyphus* as a parasitoid of *L. sativae* on alfalfa could be an interesting topic for future research.

Table 1. List of *Diglyphus* species associated with alfalfa (*Medicago sativa* L.).

<i>Diglyphus</i> species	Association with alfalfa	References
<i>Diglyphus begini</i> (Ashmead, 1904)	*	Jensen & Koehler (1970); Hutchinson <i>et al.</i> (1997)
<i>D. crassinervis</i> Erdős, 1958	†	Present study
<i>D. intermedius</i> (Girault, 1916)	*	Hendrickson & Barth (1979a)
<i>D. isaea</i> (Walker, 1838)	*	Gordh & Hendrickson (1979); Hendrickson & Barth (1979b); Drea <i>et al.</i> (1982); Hansson (1987); Askew <i>et al.</i> (2001); Lotfalizadeh <i>et al.</i> (2015)
<i>D. minoews</i> (Walker, 1838)	*	Cikman (2012)
<i>D. pachyneurus</i> Graham, 1963	*	Lotfalizadeh <i>et al.</i> (2015)
<i>D. pedicellus</i> Gordh & Hendrickson, 1979	*	De Santis (1989)
<i>D. poppoea</i> Walker, 1848	†	Present study
<i>D. propodealis</i> Szelényi, 1978	†	Present study
<i>D. pulchripes</i> (Crawford, 1912)	*	Miller & Jensen (1970); Hutchinson <i>et al.</i> (1997)
<i>D. pusztensis</i> (Erdős & Novicky, 1951)	†	Present study
<i>D. sabulosus</i> Erdős, 1951	†	Present study

* The relevant parasitoid species have been collected from alfalfa

† The relevant parasitoid species in the present study have been collected by sweeping net from alfalfa fields

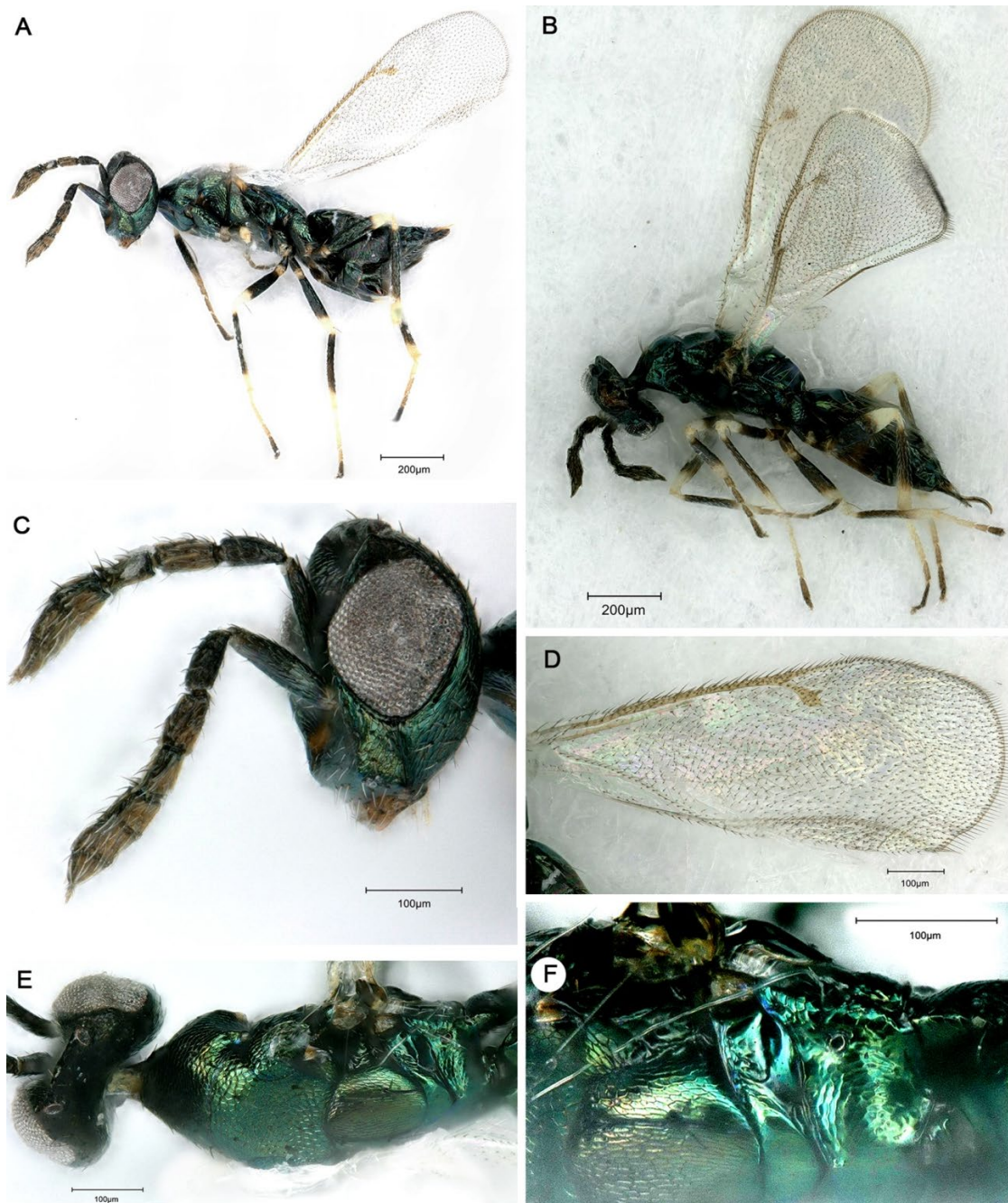


Fig. 1. *Diglyphus propodealis* Szelenyi, 1978: **A.** General habitus of female, lateral view; **B.** General habitus of male, lateral view; **C.** Head and antenna, female, lateral view; **D.** Forewing, female; **E.** Head and mesosoma, female, dorsal view; **F.** Mesoscutellum and propodeum, female, dorsal view.

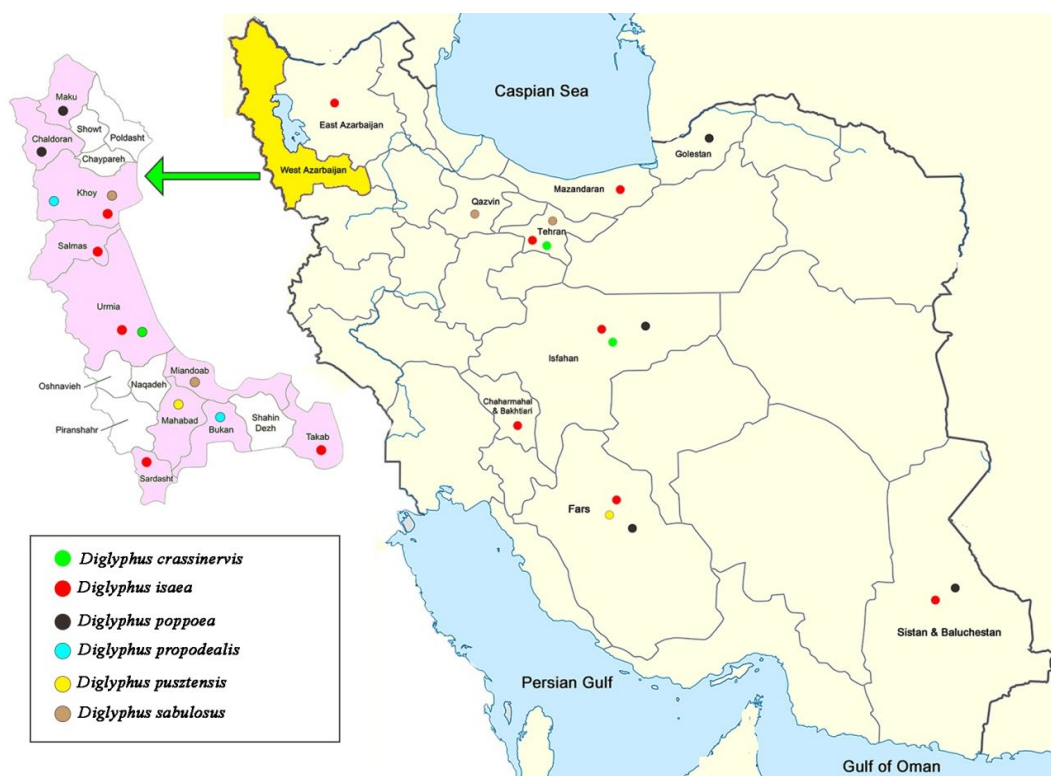


Fig. 2. Distribution map of *Diglyphus* species reported in the current research in Iran and West Azarbaijan province.

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فون زنبورهای جنس *Diglyphus* (Hymenoptera: Eulophidae) در مزارع یونجه ایران

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چکیده

با توجه به اهمیت گونه‌های جنس *Diglyphus* Walker, 1844 (Hym., Eulophidae: Eulophinae) در کنترل بیولوژیک مینوزهای برگ، یک مطالعه فونستیک در طول سال‌های 2018-2020 در مزارع یونجه استان آذربایجان غربی انجام گرفت. شش گونه به نام‌های *D. isaea* (Walker, 1838); *D. propodealis* Szelényi, 1978; *D. poppoea* Walker, 1848; *D. sabulosus* Erdős, 1951 و *D. puztensis* (Erdős & Novicky, 1951) برای فون ایران گزارش جدید می‌باشد. این گونه تاکنون فقط از محل جمع‌آوری اولیه خود، مجارستان، گزارش شده بود. اطلاعات مربوط به ویژگی‌های مورفولوژیکی مورد نیاز برای شناسایی و تصاویر *D. propodealis* فراهم شد. ارتباط این گونه با مزارع یونجه اولین گزارش ارتباط میزبانی می‌باشد. هر شش گونه گزارش شده، برای استان آذربایجان غربی جدید هستند. نقشه پراکنش گونه‌های گزارش شده فراهم شده و گونه‌های شناخته شده *Diglyphus* مرتبط با یونجه در ایران نیز مرور گردید.

کلمات کلیدی: *Medicago sativa*. Eulophinae *Diglyphus propodealis*، کنترل بیولوژیک، پارازیتوئید

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