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Diglyphus anadolucus Doğanlar, 1982 (Hym., Eulophidae) a primary larval ectoparasitoid of Anthomyiidae (Diptera), with a checklist of *Diglyphus* species in Iran

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ABSTRACT. This is the first case of host record for parasitoid wasps of the genus *Diglyphus* Walker (Hymenoptera, Eulophidae) from the Anthomyiidae (Diptera) family. This result is based on specimens of *D. anadolucus* Doğanlar, 1982 which reared from parasitized larval specimen of *Pegomya terebrans* (Rondani) as a leaf miner of cotton thistle, *Onopordum acanthium* L. (Asteraceae). *Diglyphus anadolucus* is reported from Iran for the first time. Some biological notes and morphological characteristics of *D. anadolucus* along with related photographs are given. An updated checklist of the twelve known *Diglyphus* species in Iran togther with their host associations is also provided.

Key words: biocontrol, leafminers, new host association, new record

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INTRODUCTION

Species of the genus *Diglyphus* Walker, 1844 (Eulophidae, Eulophinae) with worldwide distribution includes 41 known species, of which 31 species are distributed in the Palaearctic region (Noyes, 2019), and 11 species in Iran (Jafarlu et al., 2022). They are primary, solitary or gregarious ectoparasitoids on, mainly leaf mining Agromyzidae (Bouček & Askew, 1968), although, some species have also been reported on different hosts than Agromyzidae, especially on Lepidoptera: Gracillariidae, Lyonetiidae and Nepticulidae (Noyes, 2019). More recently, Jafarlu et al. (2022) reviewed the *Diglyphus* species in lucerne fields of Iran. Among them, there is no report on parasitizing leaf miners of the family Anthomyiidae.

MATERIAL AND METHODS

The leaf samples of *Onopordum acanthium* L. (Asteraceae) infested with a leaf miner were collected along with infected leaves from the campus of Urmia University on June 5, 2020. Samples were sorted

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and transferred to the laboratory, where they were reared inside the muslin-ventilated boxes for one week. The infested leaves were inspected under a stereomicroscope, for possible parasitoids. Once the parasitized larvae were detected, (Fig. 1), the leaves were carefully moved into a separate petri dish and kept under lab conditions, until the emergence of the adult parasitoids. The parasitoid specimens were transferred into 75% ethanol. For mounting, specimens were treated using the method suggested by Noyes (1982) so that dehydration was managed using alcoholic series of 75%, 85%, and 96%. Subsequently, for the drying phase to prevent specimens from shriveling, the CPD (critical point drying) method was utilized. The acetic acid bath was used because of the convenient utilization of the specimens during card-mounting. Rectangular cards with dimensions of 0.5 × 1.4 cm were used for card-mounting and water-soluble glue to fix specimens on the rectangular cards (Noyes, 1982). Morphological characters of specimens were examined under an Olympus SZH stereomicroscope. Images were taken with a Keyence digital microscope (VHX-5000) and edited in in Adobe Photoshop® CS6 software. The terminology followed Gibson (1997) and Yoder et al. (2010). Identification at generic and species levels was made using identification keys in Hansson and Navone (2017) and Doğanlar (1982). Identified species were deposited in Hayk Mirzayans Insect Museum, Tehran, Iran (HMIM).

RESULTS

Adult specimens of ectoparasitoid wasp were emerged after 9–10 days through rearing of their larvae on leaf miner larva. The leaf miner was identified as *Pegomya terebrans* (Rondani) (Diptera, Anthomyiidae). Cotton thistle leaf miner host plants are restricted to three species of plants belonging to tribe Cynareae (Asteraceae), including Italian thistle *Carduus pycnocephalus* L. and two species of cotton thistle namely *Onopordum acanthium* L. and *O. leptolepis* DC. in Urmia environs (see Karimpour et al., 2021).

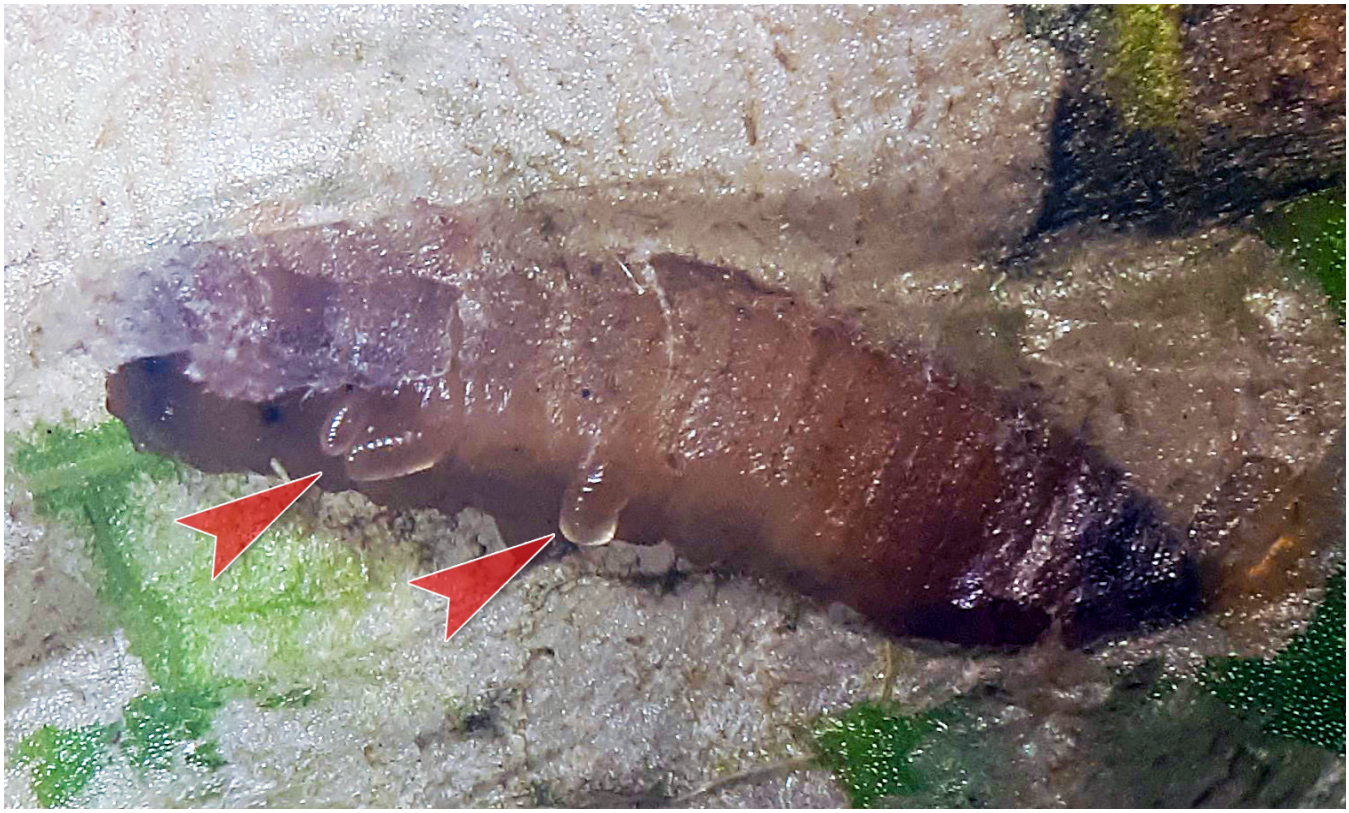

Figure 1. The larvae of *Diglyphus anadolucus* Doğanlar, feeding on larva of *Pegomya terebrans* (Rondani) as an ectoparasitoid.

Taxonomic hierarchy

Class Insecta Linnaeus, 1758

Order Hymenoptera Linnaeus, 1758

Superfamily Chalcidoidea Latreille, 1817

Family Eulophidae Westwood, 1829

Subfamily Eulophinae Westwood, 1829

Genus *Diglyphus* Walker, 1844

Diglyphus Walker, 1844:409. Type species: *Cirrospilus chabrias* Walker, 1838:451, designated by monotypy.

Diagnosis. Both female and male with two funiculars and three clavomeres, males with unbranched flagellomeres. Submarginal vein smoothly joining to parastigma and having more than 3 dorsal setae. Mesoscutellum with submedian grooves, Propodeum without plicae. Gaster sessile, body medium sized, color dark and metallic.

Diglyphus anadolucus Doğanlar, 1982

Diglyphus anadolucus Doğanlar, 1982:75–78. ♂♀, Holotype ♀, AUET, Türkiye. Not examined.

(Figs 2A–E)

Material examined. 6 ♀♀; 14–15, June 2020; ex. *Pegomya terebrans* (Rondani) (Diptera, Anthomyiidae) on *Onopordum acanthium* DC. (Asteraceae); Urmia, Urmia University campus, 37°39'14''N, 44°58'35''E, 1360 m a.s.l., leg. Y. Karimpour.

Diagnosis — *Female*. Body metallic green with golden reflections on mesoscutellum (Figs 2A, 2C, 2D) Pedicel and flagellum blackish. Scape white with anterior 0.36 in dark brown color (Fig. 2B). Femora and tibiae white, except for the hind femur which is dark brown at 1/3 its base (Fig. 2A). Antenna with first funicular 2.13× and second funicular 1.50× as long as wide; pedicel 0.63× as long as length of first flagellomere; and first funicular 1.17× as long as second funicular (Fig. 2B). Costal cell with a complete row of setae on ventral surface and with 11 setae on the dorsal surface along the terminal part of its anterior margin (Fig. 2E)

In the key provided by Hansson and Navone (2017), based on specimens obtained from Italy and Russia the first funicular of female antennae is 1.6× and second one is 1.3× as long as wide. While in the specimens of current study, they are 2.13× and 1.50× as long as wide, respectively (Fig 2B). This corresponds to the characteristics of the corresponding holotype (Doğanlar, 1982). Therefore, it is suggested that in the identification key published by Hansson and Navone (2017), the scales mentioned for first and second funiculars of female antennae be modified as follows: the first funicular 1.6–2.2× and second funicular 1.3–1.6× as long as wide.

Distribution in Iran. West Azarbaijan province, Urmia (present study).

General distribution. Iran (**New record**), Italy, Russia (Hansson & Navone, 2017), and Türkiye (Doğanlar, 1982).

DISCUSSION

The genus *Diglyphus* includes 41 species worldwide (Noyes, 2019), which are mainly the ecto-parasitoids of leafmining agromyzids (Diptera, Agromyzidae) (Bouček & Askew, 1968). According to the Hansson and Navone (2017) all European and Asian species of *Diglyphis* are associated with the various genera of Agromyzidae, as even the citrus leafminer, *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera, Gracilariidae) (González Tirado et al., 1996). Therefore, this is the first confirmed record indicating the association of a *Diglyphus* species with the family Anthomyiidae. *Diglyphus anadolucus* was originally described from the material reared from an unknown leaf miner on Alfalfa

(Doğanlar, 1982), later it was reared from *Agromyza abiens* Zetterstedt, 1848 as a leaf miner of *Echium vulgare* L. (Boraginaceae) in Italy (Hansson & Navone, 2017). Among the 41 species of *Diglyphus* known worldwide, none of them have been reported as the primary parasitoid of Anthomyiidae (Noyes, 2019).

The plant association and primary hosts of the *Diglyphus* species reported in Iran are presented in Table 1. Including a new record for the Iranian fauna in this study, so far 12 species of the genus *Diglyphus* have been reported from Iran.

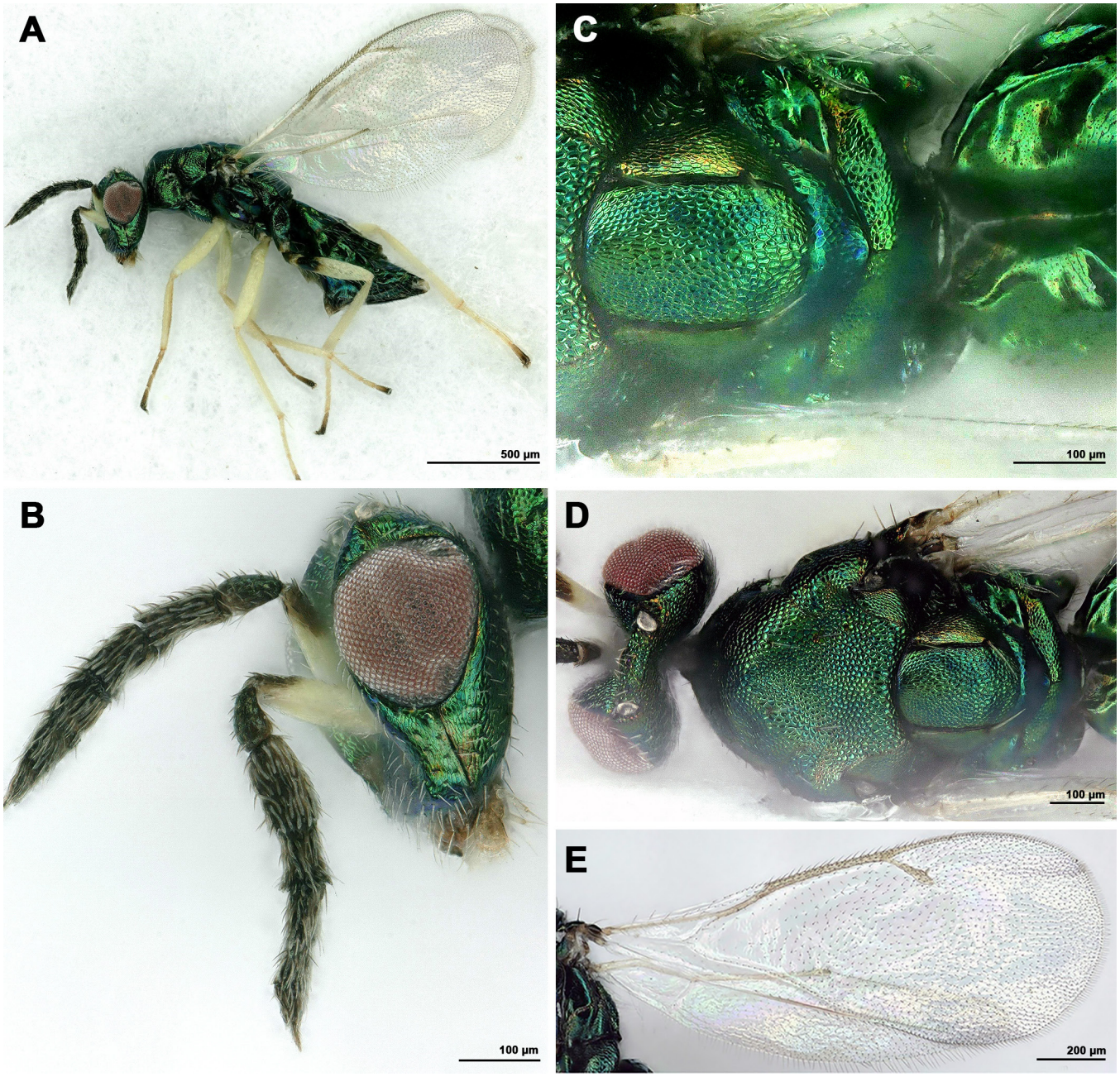

Figure 2. *Diglyphus anadolucus*, female: **A.** General habitus, lateral view; **B.** Head and antenna, lateral view; **C.** Mesoscutellum and propodeum, dorsal view; **D.** Head and mesosoma, dorsal view; **E.** Fore wing.

Table 1. List of *Diglyphus* species and their host association in Iran.

Diglyphus species	Collecting data			References
	Locality (province)	Host plants	Primary hosts	
Diglyphus anadolucus Doğanlar, 1982	West Azarbaijan	*Onopordum acanthium*	*Pegomya terebrans*	Present study
D. propodealis Szelényi, 1978	West Azarbaijan	-	-	Jafarlu et al. (2022)
D. chabrias (Walker, 1838)	Fars	*Lactuca serriola*	*Liriomyza trifolii*	Fallahzadeh et al. (2006)
	Lorestan	-	*Syringopais temperatella*	Yefremova et al. (2007)
D. crassinervis Erdös, 1958	Isfahan	-	-	Ghahari & Yefremova (2013)
	Tehran	*Cucumis sativus* *Lycopersicon esculentum* *Citrullus lanatus* *Phaseulus vulgaris*	*Liriomyza sativae* *Liriomyza trifolii*	Asadi et al. (2006)
D. isaea (Walker, 1838)	Chaharmahal & Bakhtiari	-	-	Yefremova et al. (2007)
	East Azarbaijan	*Medicago sativa*	*Chromatomyia horticola* *Liriomyza sativae* *Liriomyza trifolii*	Lotfalizadeh et al. (2015)
	Fars	-	*Liriomyza trifolii*	Dousti et al. (2008)
	Golestan, Isfahan, Mazandaran	-	-	Ghahari & Yefremova (2013)
	Sistan & Baluchestan	*Triticum aestivum* *Helianthus annus* *Silybum* sp. *Brassica rapa*	*Chromatomyia nigra* *Chromatomyia horticola*	Shahreki et al. (2012)
	Tehran	*Cucumis sativus* *Lycopersicon esculentum* *Citrullus lanatus* *Phaseulus vulgaris*	*Liriomyza sativae* *Liriomyza trifolii*	Asadi et al. (2006)
D. minoeus (Walker, 1838)	Isfahan	-	-	Yefremova et al. (2007)
D. pachyneurus Graham, 1963	East Azarbaijan	*Medicago sativa*	*Chromatomyia horticola* *Liriomyza trifolii*	Lotfalizadeh et al. (2015)
D. poppoea Walker, 1848	Fars	*Triticum aestivum*	*Agromyza ambigua*	Dousti et al. (2008)
	Golestan, Isfahan	-	-	Ghahari & Yefremova (2013)
	Sistan & Baluchestan	*Brassica rapa* *Cucumis sativus*	*Chromatomyia horticola*	Shahreki et al. (2012)
D. pulchripes (Crawford, 1912)	Fars	*Medicago sativa*	*Liriomyza trifolii*	Dousti et al. (2008)
D. pusztensis (Erdös & Novicky, 1951)	Fars	Sweeping in wheat fields	-	Hesami et al. (2010)
D. sabulosus Erdös, 1951	Tehran	-	-	Farahbakhsh (1961)
D. scapus Yefremova, 2008	Mazandaran	Sweeping in pastures	-	Hesami et al. (2006)

(-) Collected by sweeping net, and host plant/primary host is unknown.

AUTHOR'S CONTRIBUTION

The authors confirm their contribution in the paper as follows: Y.Karimpour: Collection of the specimens, recording the biological data in Urmia. All authors confirmed their contribution in identification the specimens, sorting and deposition in the collection, preparing the diagnostic characters, preparation of the draft, corrections on the final contents of the manuscript.

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AVAILABILITY OF DATA AND MATERIAL

The specimens listed in this study are deposted in Hayk Mirzayans Insect Museum, Tehran, Iran and are available from the curator, upon request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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زنبور *Diglyphus anadolucus* Doğanlar, 1982 (Hym., Eulophidae) پارازیتویید خارجی و اولیه مرحله لاروی دوبالان خانواده Anthomyiidae، و فهرست گونه‌های جنس *Diglyphus* در ایران

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چکیـــده: این اولین مورد از ثبت میزبان برای زنبورهای پارازیتویید جنس *Diglyphus* Walker (Hymenoptera, Eulophidae) از دوبالان خانواده Anthomyiidae است. این نتیجه، براساس نمونه‌هایی از زنبور *D. anadolucus* Doğanlar, 1982 بود که از پرورش لاروهای این زنبور روی لاروهای مگس *Pegomya terebrans* (Rondani) به عنوان مگس مینوز خارپنبه، *Onopordum acanthium* L. (Asteraceae) به دست آمدند. زنبور *D. anadolucus* برای اولین بار از ایران گزارش می‌شود. برخی نکات زیستی و ویژگی‌های ریخت‌شناسی این زنبور به همراه تصاویر مربوطه معرفی شد. فهرست به‌روز ۱۲ گونه از جنس *Diglyphus* و اطلاعات موجود از روابط میزبانی آنها در ایران نیز ارایه شد.

واژگـــان کلیدی: مهار زیستی، مینوز برگ، روابط میزبانی جدید، گزارش جدید