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Hyssopus geniculatus (Hartig) (Hymenoptera, Eulophidae) a parasitoid of *Anarsia eleagnella* Kuznetsov (Lepidoptera, Gelechiidae) in Iran

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ABSTRACT. Through a survey conducted in West Azarbaijan province, northwestern Iran in 2022, infested fruits of *Elaeagnus angustifolia* L. (Elaeagnaceae) were collected. *Anarsia eleagnella* Kuznetsov, 1957 (Lepidoptera: Gelechiidae) was obtained from laboratory rearing. A parasitic wasp was collected in the rearing container that was identified under *Hyssopus geniculatus* (Hartig, 1838) (Hymenoptera: Eulophidae). This parasitoid is a primary ectoparasitoid and a new record of *H. geniculatus* on *A. eleagnella*. Also, the association of *H. geniculatus* with *E. angustifolia* and the family Elaeagnaceae is new. Notes on the diagnostic characters of *H. geniculatus* are provided and illustrations of *A. eleagnella* and *H. geniculatus* are presented. The feeding behaviour of *A. eleagnella* larvae is preliminarily studied. Finally, the colour variations of the collected *H. geniculatus* specimens compared to the published literature are discussed.

Key words: *Anarsia eleagnella*, *Elaeagnus angustifolia*, Eulophinae, oleaster, Russian olive

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INTRODUCTION

Russian olive or oleaster, *Elaeagnus angustifolia* L. (Elaeagnaceae) has many benefits and is widely used (Little, 1961; Borell, 1962; Bartha & Csiszár, 2008). The genus *Anarsia* Zeller, 1839 (Lepidoptera, Gelechiidae) has about 100 described species in the world (Ueda, 1997; Ponomarenko, 2009; Park & Bae, 2017) and so far, five species of *Anarsia* have been reported in Iran (Rajaei et al., 2023). This genus is distributed all over the world except the Antarctic region (Ponomarenko, 1997), but its main distribution is in the Palaearctic and Oriental regions (Park & Bae, 2017). *Anarsia eleagnella* Kuznetsov,

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1957 is distributed in the Palaearctic region in European part of Russia, Romania, Ukraine, Afghanistan, Kazakhstan, Transcaucasia (Ponomarenko, 1997), Iran (Asadi et al., 2014). Plant hosts of *A. eleagnella* include *Elaeagnus* spp. and *Hippophae* spp., both belonging to the family Elaeagnaceae (Ponomarenko, 1997). The only parasitoid belonging to the superfamily Chalcidoidea that so far was reported against *A. eleagnella* is *Copidosoma varicorne* Nees, 1834 (Hymenoptera: Encyrtidae), a primary endoparasitoid of the egg-larvae of lepidopterans (Trjapitzin, 1989; Guerrieri & Noyes, 2005).

Considering the ecological, medical, and economic importance of the Russian olive, as well as the importance of *A. eleagnella* damage on it, the objectives of this research are (**1**) report of a new parasitoid of *A. eleagnella* which probably can be a potential agent for biological control, (**2**) report of a new plant association for the respective parasitoid, (**3**) study of the feeding behaviour of *A. eleagnella* larvae on Russian olive fruits, and (**4**) compare of the colour variations of the Iranian *H. geniculatus* specimens with the specimens in the published literature for other parts of the world.

MATERIAL AND METHODS

The current research was conducted in 2022 in West Azarbaijan province, located in the northwest of Iran, in Khoy and Urmia counties. In this study, from August to November, Russian olive fruits were collected from five gardens. Infested fruits with holes in the rind (Fig. 1C) were collected. The collected fruits were transferred to the laboratory in ziplock plastic bags. Then, the infested fruits were placed in 12 Petri dishes with a diameter and depth of 7.8 × 1.2 cm (Fig. 1C), and kept under ambient laboratory conditions. The adult moth and parasitoid wasps that emerged from the larvae were collected using a camel's hair brush (Figs 1A–1B, 2A–2C). The moths were mounted and parasitoid wasps were transferred to 75% alcohol. The mounted lepidopterans were preliminarily identified and later confirmed by Dr. S. Sinev (Zoological Institute of the Russian Academy of Sciences, St Petersburg, Russia). Parasitoid specimens were dehydrated using an alcohol series of 75, 85, and 96%. For the drying phase, these specimens were placed in acetone for 24 hours, then they were fixed on rectangular point cards using water-soluble glue (Noyes, 1982).

Examining the morphological characters of the specimens and identifications were done using an Olympus SZH stereomicroscope. The identified parasitoid wasps were confirmed by Dr. C. Hansson (Biological Museum, Lund University, Lund, Sweden; Natural History Museum, London, UK). An Olympus SZH Stereomicroscope and a mobile phone camera (with 13 mega-pixels resolution) were used to prepare the photographs of *A. eleagnella*. Illustrations of the parasitoid wasp were obtained using a Keyence® VHX-5000 digital microscope. Then these photos were edited and inserted in the plates using Adobe Photoshop® CC software (2015.0.0 Release). Morphological terminology followed by Gibson (1997) and Yoder et al. (2010). All materials are deposited in the insect collection of the Hayk Mirzayans Insect Museum (HMIM), Iranian Institute of Plant Protection, Tehran, Iran.

RESULTS

By rearing the active larvae inside the fruits of Russian olive (*Elaeagnus angustifolia* L.) (Elaeagnaceae), the emerged lepidopteran was identified as *Anarsia eleagnella* Kuznetsov, 1957 (Lepidoptera, Gelechiidae). Larvae of *A. eleagnella* (Fig. 1D) make holes in the epicarp and feed from the mesocarp. These larvae create tunnels in the mesocarp and advance to the endocarp. However, they are not able to penetrate the endocarp and access the seed. They are only able to feed around the endocarp. Larvae start webbing from young instars and attach the mesocarp tissues and excreta in the feeding places and the tunnels they have created. Also, due to the presence of this network of webs in their feeding sites (Fig. 1C), the emerged parasitoid wasps of *H. geniculatus* were extensively covered with webs. Young larvae are light in colour and gradually change to light brown in later instars. The fully-grown larvae stop feeding and use the threads they have spun to form a puparium inside the mesocarp and become pupa (Fig. 1C). The parasitoid wasp emerged from the larvae of *A. eleagnella* was identified as *Hyssopus geniculatus* (Hartig, 1838) (Hymenoptera, Eulophidae). This is a new host association for *H. geniculatus*. Also, the association of *H. geniculatus* with *E. angustifolia* is a new plant association.

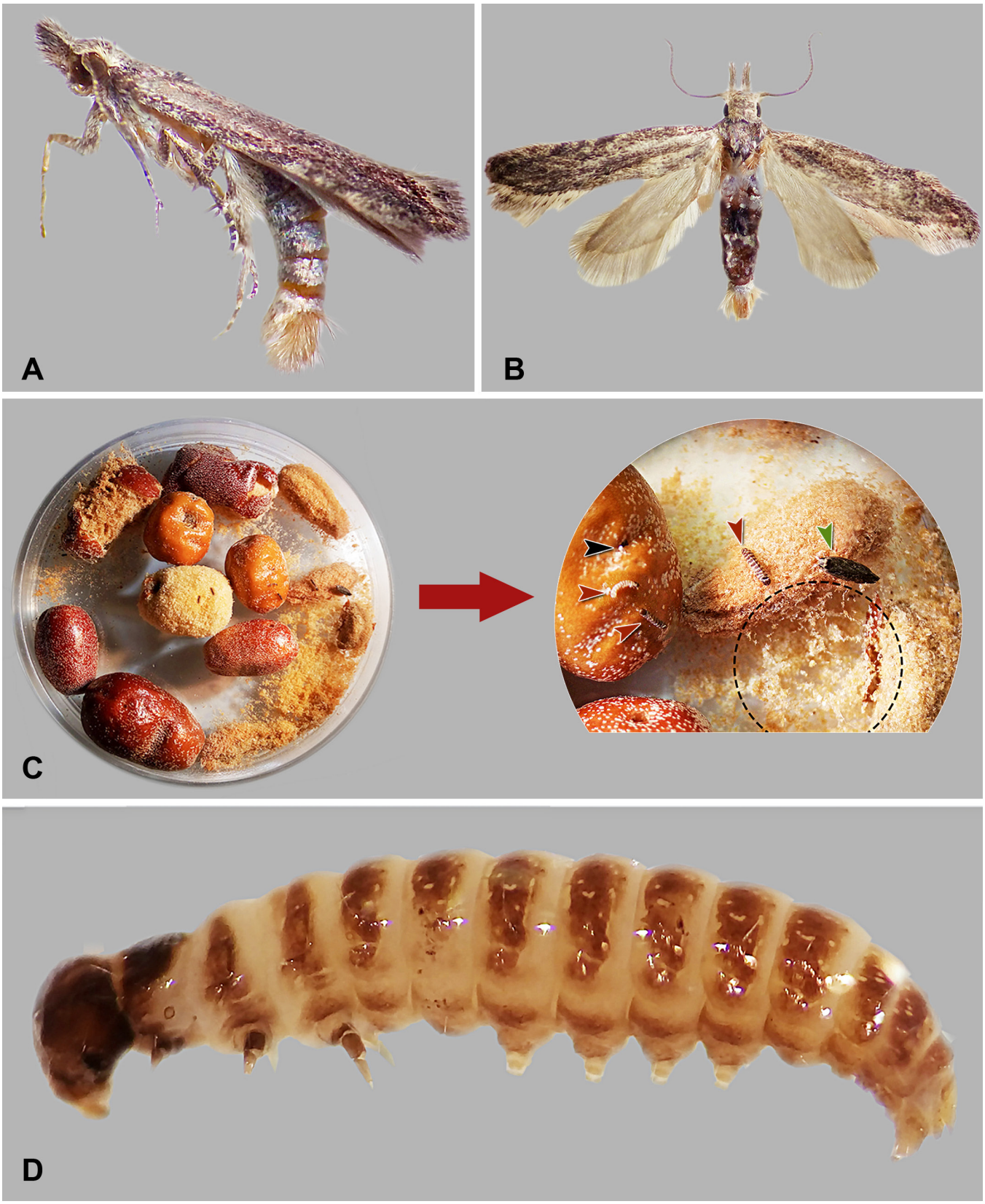

Figure 1. *Anarsia eleagnella* Kuznetsov, 1957. **A.** Adult in lateral view; **B.** Adult in dorsal view; **C.** Growth chamber including damaged and non-damaged fruits of Russian olive along with emerged larvae and adult; **D.** Fully-grown larva in latero-dorsal view. (Black arrow points to a hole made by the larva on the epicarp, green arrow to adult, red arrows point to larvae of different instars; dashed circle indicates the mesocarp tissues sticked together by the threads of the larvae).

Taxonomic hierarchy

Order Hymenoptera Linneaus 1758

Superfamily Chalcidoidea Latreille, 1817

Family Eulophidae Westwood, 1829

Subfamily Eulophinae Westwood, 1829

Genus *Hyssopus* Girault, 1916

Type species: *Hyssopus thymus* Girault, 1916; **Syn.:** *Hyssopiscus* Ghesquière, 1946; *Crataepoides* Masi, 1955.

Diagnosis. Body black to blackish green with metallic luster (Figs 2, 3). Notauli complete; mesoscutum hemispherical with two pairs of setae on the mid lobe (Fig. 3C); mesosoma dorsally with fine reticulation on its entire surface (Figs 3C, 3F); mesoscutellum with distinct and complete submedian grooves that bend inward in the posterior part and nearly meeting each other. Propodeum with a simple median carina and without plicae or costula (Figs 3C, 3F). Antenna with four funiculars in both male and female (Fig. 2); anterior margin of clypeus not produced and only slightly rounded (Fig 3B); pronotum long and semi-globose with rounded front part (Figs 3A, 3C, 3D).

Remarks. The genus *Hyssopus* has 23 species worldwide, 12 of which are distributed in the Palaearctic region (Noyes, 2019), and two in Iran (Hesami et al., 2018). Species of this genus are primary ectoparasitoids of microlepidopterans larvae such as Coleophoridae, Gelechiidae, Pyralidae, and Tortricidae (Bouček, 1988), and in general, its primary hosts are lepidopterous larvae in concealed situations (Burks, 2003).

***Hyssopus geniculatus* (Hartig, 1838)** (Figs 2, 3)

Eulophus geniculatus Hartig, 1838, *Hyssopus geniculatus* (Hartig, 1838), *Crataepoides russoi* Zinna, 1955, not examined.

Material examined. IRAN - West Azarbaijan province: 3 ♀♀, 2 ♂♂ (HMIM): Khoy; 38°42'30.648" N, 44°54'1.540" E; Elev. 1414 m a.s.l.; 19.viii.2022; reared on *Anarsia eleagnella* Kuznetsov, 1957 (Lepidoptera, Gelechiidae) emerged from Russian olive (*Elaeagnus angustifolia* L.); M. jafarlu leg. 3 ♀♀ (HMIM): Khoy; 38°35'38.805" N, 44°48'32.172" E; Elev. 1293 m a.s.l.; 03.ix.2022; same data as for preceding; 2 ♀♀, 1 ♂ (HMIM): Urmia; 37°40'5.358" N, 45°0'43.846" E; Elev. 1340 m a.s.l.; 08.xi.2022; same data as for preceding; 18 ♀♀, 10 ♂♂ (HMIM): Urmia; 37°33'13.648" N, 45°8'22.388" E; Elev. 1305 m a.s.l.; 16.x.2022; same data as for preceding; M. Hassan-Pashai-Mehr leg.

Diagnosis — Female. Head and mesosoma blackish green with metallic luster (Figs. 2A, 3A–D, F); antenna bronzy (Fig. 2); legs bronzy to amber; basal parts of coxae concolourous with mesosoma (Fig. 2A, 3A); metasoma fuscous to bronzy (Fig. 2A, 3G). Antenna of the female with a lower placoid sensillae than that of *Hyssopus nigritulus* (Zetterstedt, 1838) (Fig. 2A, 2B). Thorax flat dorsally (Fig. 2A, 3A); mesoscutum with fine reticulation (Fig. 3C); mesoscutellum smooth and shiny, sometimes alutaceous anteriorly (Figs 3C, 3F). Fore wing with marginal vein 3× as long as stigmal vein; submarginal vein with 7 setae dorsally; speculum small (Fig. 2A, 3E). Gaster oval and longer than the length of the thorax and propodeum together (Fig. 2A, 3G).

Male. Similar to female except enlarged and more globular head; antennal scape inflated with fewer placoid sensillae; gaster squatter than that of female (Figs 2C, 2D).

Primary hosts. Based on our findings, *H. geniculatus* is a primary ectoparasitoid of *A. eleagnella* larvae. Generally, *H. geniculatus* is an ectoparasitoid of lepidopterous larvae (Bouček & Askew, 1968; Trjapitzin, 1978; Ermolaev et al., 2019), and additionally, two coleopterous species from Scolytidae and one dipterous species from Cecidomyiidae have been reported as its primary hosts (Bouček & Askew, 1968). Its lepidopterous hosts are Gelechiidae, Gracillariidae, Pyralidae, and Tortricidae (Bouček & Askew, 1968; Bouček, 1977; Trjapitzin, 1978; Yefremova et al., 2007).

Distribution. Afrotropical: Yemen (Yefremova, 2007); Palaearctic: Europe, Israel, Japan, Korea, Peoples' Republic of China, Russia, Tajikistan, Turkey, Turkmenistan (Noyes, 2019), Iran (Yefremova et al., 2007; Ebrahimi et al., 2009).

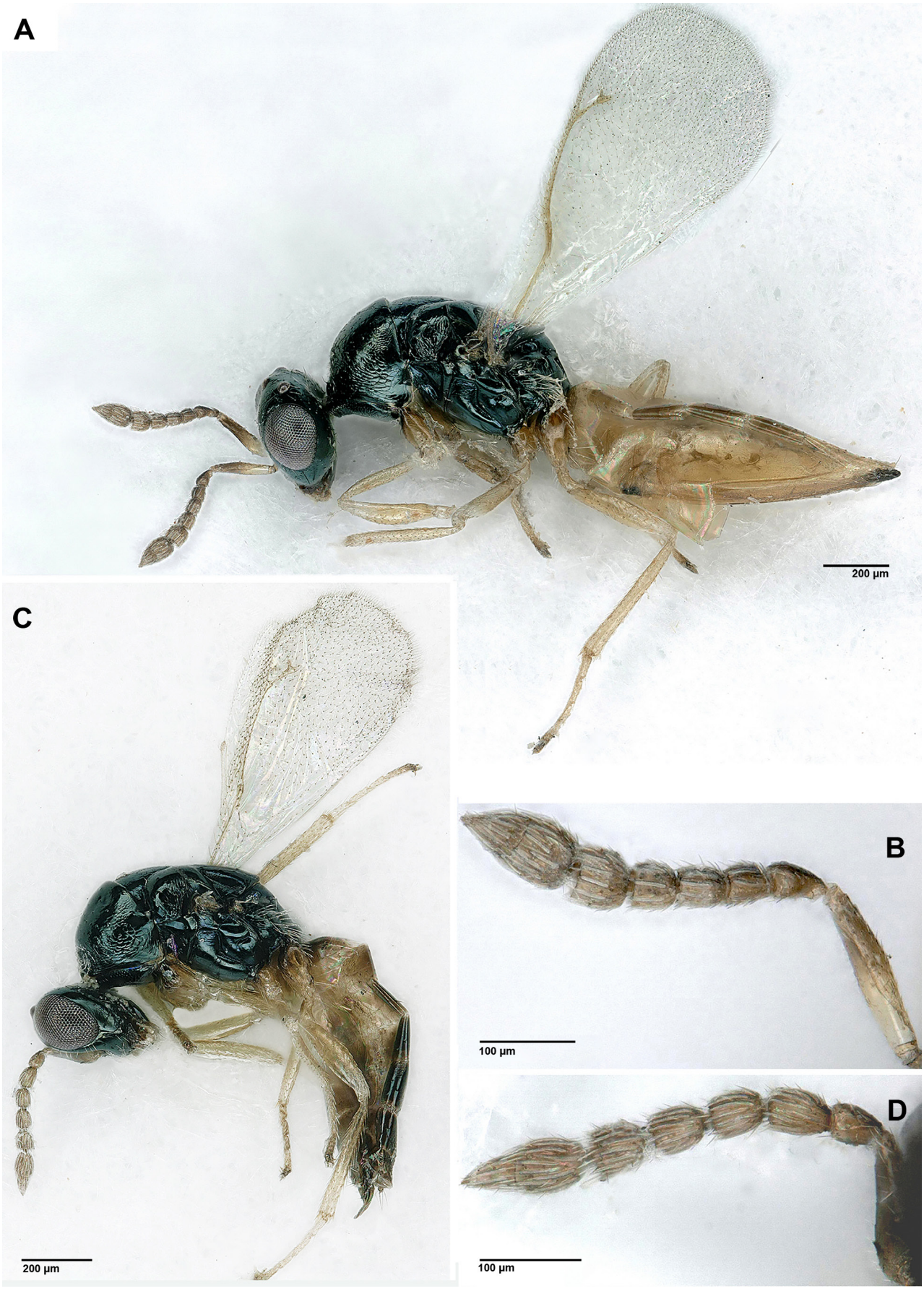

Figure 2. *Hyssopus geniculatus* (Hartig, 1838). **A.** Female habitus, lateral view; **B.** Female antenna; **C.** Male habitus, lateral view; **D.** Male antenna.

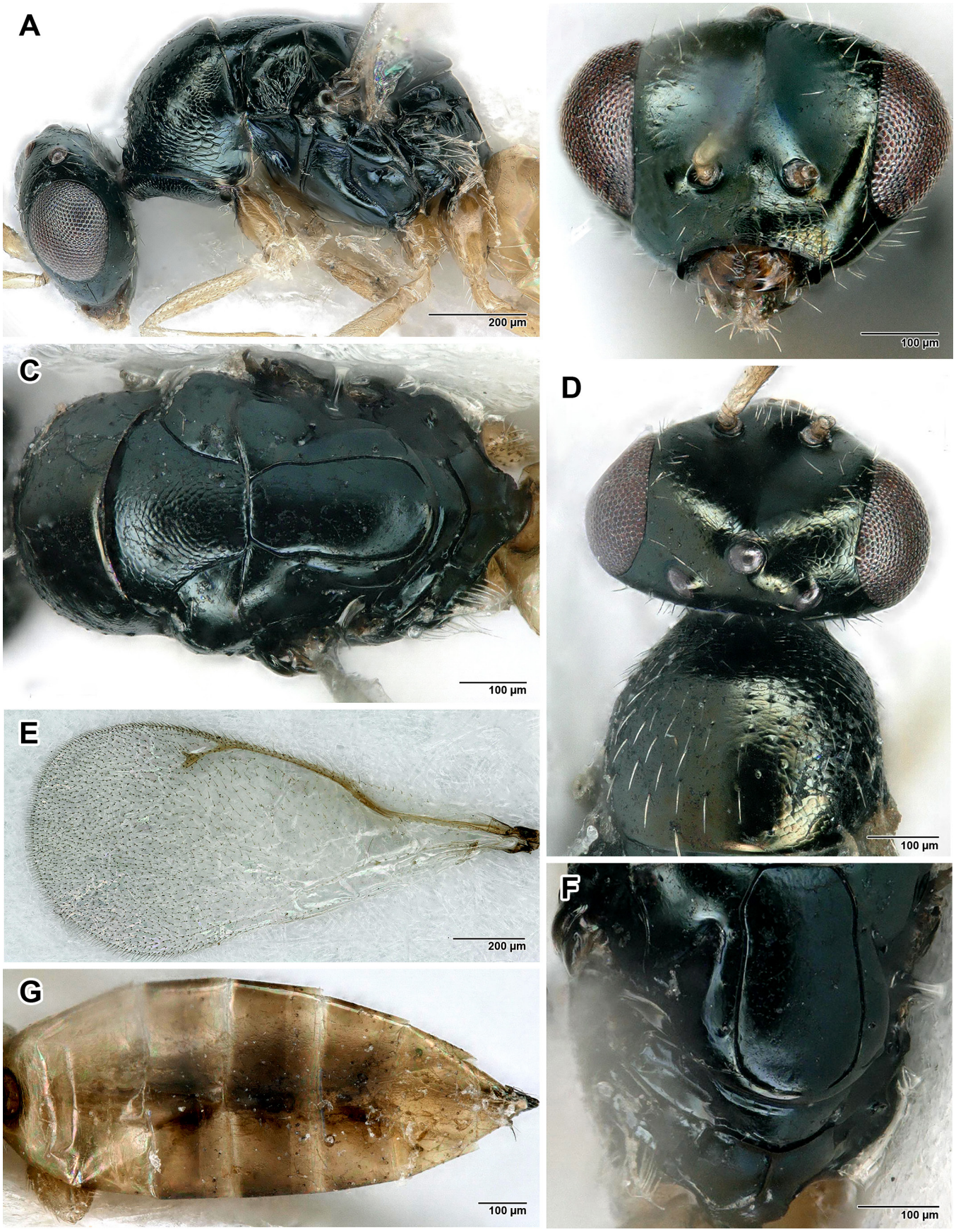

Figure 3. *Hyssopus geniculatus* (Hartig, 1838), female. **A.** Head and mesosoma, lateral view; **B.** Head, frontal view; **C.** Mesosoma, dorsal view; **D.** Head and pronotum, dorsal view; **E.** Fore wing; **F.** Mesoscutellum and propodeum, dorsal view; **G.** Metasoma, dorsal view.

DISCUSSION

So far, the only eulophid that has been reported from the Russian olive is *Tamarixia poddubnyi* (Kostjukov, 1978) (Zuparko et al., 2011). Hence, the report of *Hyssopus geniculatus* in the present study is the second eulophid report associated with *Elaeagnus angustifolia*. As for the plant association, so far, *H. geniculatus* has been reported only associated with *Centaurea scabiosa* L. (Asteraceae) (Vidal, 1993), and several species of the family Pinaceae (Noyes, 2019). Therefore, its association with *E. angustifolia* and the family Elaeagnaceae is new. *Hyssopus geniculatus* is generally a gregarious larval-pupal ectoparasitoid (Bouček & Askew, 1968). Yefremova and Mishchenko (2008) reported it as a solitary ectoparasitoid. Based on our findings, this wasp is a larval ectoparasitoid of *A. eleagnella*. So far, from the Gelechiidae family apart from *A. eleagnella*, three species *Exoteleia* sp., *Exoteleia dodecella* (L.), and *Metzneria* sp. have been reported as the primary hosts of *H. geniculatus* (Bouček & Askew, 1968; Bouček, 1977; Trjapitzin, 1978; Vidal, 1993). Scape of the male antenna in *H. geniculatus* has many dense and fine MPS (Figs 2C, 2D), which this feature is only seen in *Ceranisus* and *Hyssopus* species, and is not present in the other eulophids (Doğanlar & Doğanlar, 2013). Also, by comparing the specimens of *H. geniculatus* reported in the present study with the published literature, some differences in colour patterns are observed: (**1**) Head and mesosoma black (Zinna, 1955), while our studied specimens have a green metallic luster (Figs 2A, 2C, 3A–3C); (**2**) Legs with tibiae and apical third of femora fuscous and the rest black (Zinna, 1955; Askew, 1964), while in our collection, legs generally are fuscous with coxae basally concolourous with mesosoma (Figs 2A, 2C, 3A).

Despite the many benefits of the Russian olive, this species due to its adaptive features quickly reproduces and becomes a dominant plant. Hence, in parts of the United States of America and in some other countries, it is considered a negative factor. Consequently, in places with these conditions, this species is a target for biological control (Shafroth et al., 1995; Katz & Shafroth, 2003; Bartha & Csiszár, 2008). Therefore, *A. eleagnella* and *Aceria eleagnicola* Farkas, 1963 (Acariformes, Eriophyidae) were considered the most promising agents for the biological control of Russian olive (Gaskin et al., 2019). On the contrary, in Iran, it is a useful tree with minimum water requirements and compatibility with poor soils. Hence, the effort is generally to preserve it. Furthermore, since *H. geniculatus* is reported for the first time from *A. eleagnella* larvae, many aspects of its biology and parasitic behaviour against lepidopterous larvae are unknown and require future studies.

AUTHOR'S CONTRIBUTION

The authors confirm their contribution to the paper as follows: M. Jafarlu: The main researcher, the methodologist, introduction writer, discussion writer, taxonomic identifier, data collector, drafting; M. Hassan-Pashai-Mehr: The assistant researcher, data collector, drafting, the methodologist; H. Lotfalizadeh: The main researcher, conceptualization of work, project supervisor, technical review of the manuscript, the methodologist, taxonomic identifier, drafting; S. Asghari-Tazehkand: The assistant researcher, data collector, drafting, the methodologist; Y. Karimpour: The assistant researcher, discussion writer, drafting. The authors read and approved the final version of the manuscript.

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AVAILABILITY OF DATA AND MATERIAL

The specimens listed in this study are deposited in the insect collection of the Hayk Mirzayans Insect Museum (HMIM), Iranian Institute of Plant Protection, 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 *Hyssopus geniculatus* (Hartig) (Hymenoptera, Eulophidae) پارازیتویید *Anarsia eleagnella* (Lepidoptera, Gelechiidae) Kuznetsov در ایران

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چکیده: طی پژوهش صورت‌گرفته در سال ۱۴۰۱ در استان آذربایجان‌غربی در شمال‌غربی ایران، میوه‌های آفت‌زده سنجد، *Elaeagnus angustifolia* L. (Elaeagnaceae) جمع‌آوری شده و در پرورش آزمایشگاهی، *Anarsia eleagnella* Kuznetsov, 1957 (Lepidoptera, Gelechiidae) از آن‌ها خارج شد. همچنین، یک گونه زنبور پارازیتویید از خانواده Eulophidae (Hymenoptera, Chalcidoidea) در ظروف پرورش ظاهر شدند که تحت نام *Hyssopus geniculatus* (Hartig, 1838) (Hymenoptera, Eulophidae) شناسایی گردید. این زنبور، پارازیتویید خارجی اولیه لاروها بوده و *A. eleagnella* به عنوان میزبان اولیه زنبور *H. geniculatus* یک گزارش جدید است. همچنین، ارتباط *H. geniculatus* با *E. angustifolia* و خانواده Elaeagnaceae یک یافته جدید است. به علاوه، ویژگی‌های مورفولوژیک مهم در شناسایی *H. geniculatus* فراهم شده و تصاویر *A. eleagnella* و *H. geniculatus* ارایه شد. رفتار تغذیه‌ای لاروهای *A. eleagnella* به‌صورت مقدماتی مورد مطالعه قرار گرفت. همچنین، تغییرات رنگی نمونه‌های *H. geniculatus* جمع‌آوری‌شده با منابع منتشر شده بحث شدند.

واژگان کلیدی: *Anarsia eleagnella*، *Elaeagnus angustifolia*، Eulophinae، سنجد، زیتون روسی