J. Insect Biodivers. Syst. 10 (1), 133–142

https://jibs.modares.ac.ir

OPEN ACCESS

Tarbiat Modares University Press | Research Article | https://doi.org/10.61186/jibs.10.1.133 | **ISSN**: 2423-8112

Entomological Society of Iran | Taxonomy | *https://zoobank.org/urn:lsid:zoobank.org:389B300E-4CE2-49D6-B093-F3977EB559E9*

Baryscapus evonymellae (Bouché, 1834) (Hymenoptera: Eulophidae), a hyperparasitoid of *Leucoma wiltshirei* Collenette, 1938 (Lepidoptera: Erebidae) in Iran

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ABSTRACT. *Baryscapus evonymellae* (Bouché, 1834) (Hymenoptera, Eulophidae) was rediscovered on *Leucoma wiltshirei* Collenette, 1938 (Lepidoptera, Erebidae) attacked by different parasitoids. This species had already been reported under different names; therefore, its inaccurate identifications were corrected. It was reared as a larval and pupal hyperparasitoid of two important primary parasitoids of *L. wiltshirei* including *Brachymeria tibialis* Steffan, 1958 (Hymenoptera, Chalcididae), and *Dolichogenidea persica* Abdoli, Mohammadi, Sedaratian-Jahromi & Farahani, 2023 (Hymenoptera, Braconidae). The last parasitoid-hyperparasitoid association is new. Its morphological characters were illustrated and its biological data and main characteristics were discussed.

Key words: Braconidae, biocontrol, Chalcididae, forest, parasitoid, pest, Zagros

Received: 24 October, 2023

Accepted: 14 January 2024

Published: 19 January, 2024

Subject Editor: Majid Fallahzadeh

Citation: Ehteshami, F., Jafarlu, M., Mohammadi, H., Sedaratian-Jahromi, A., Iranipour, Sh., Lotfalizadeh, H. & Kiany, M. (2024) *Baryscapus evonymellae* (Bouché, 1834) (Hymenoptera, Eulophidae), a hyperparasitoid of *Leucoma wiltshirei* Collenette, 1938 (Lepidoptera, Erebidae) in Iran. *Journal of Insect Biodiversity and Systematics*, 10 (1), 133–142.

INTRODUCTION

Wiltshire's white, *Leucoma wiltshirei* Collenette, 1938 (Lepidoptera, Erebidae) is a key pest of *Quercus brantii* Lindl. It is distributed in Iran (Abai, 1980–1981, 1999; Moradi et al., 2021; Rajaei et al., 2023), Iraq and Turkey (Kemal et al., 2013). Its larvae defoliate oak trees throughout the growing season, causing heavy damage to this host plant. In recent years, pest outbreaks with irreversible damage have been reported in the southern Zagros mountains forest-steppe, Iran. Different life stages of *L. wiltshirei* are

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attacked by some parasitoids (Mirzayans & Abai, 1974; Haeselbarth, 1983; Mozaffari et al., 1997; Ghassemi & Alhoseini, 2010; Lotfalizadeh et al., 2012; Abdoli et al., 2023; Ehteshami et al., 2023). Noyes (2019) listed chalcidoid species from two families, Chalcididae and Pteromalidae associated with *L. wiltshirei*. Most recently, *Ooencyrtus ocneriae* Hayat & Mehranjad, 2018 (Hymenoptera, Encyrtidae) (Ehteshami et al., 2023) and *Dolichogenidea persica* Abdoli, Mohammadi, Sedaratian-Jahromi & Farahani, 2023 (Hymenoptera, Braconidae) were reported from egg and larval stages, respectively. Information about the biology and ecology of these parasitic wasps is not available (Mirzayans & Abai, 1974; Mozaffari et al., 1997).

Hyperparasitoids known as insects in the fourth trophic level parasitize the larvae or pupae of primary parasitoids (Sullivan & Völkl, 1999). These groups challenge rules on nutrient use efficiency in trophic chains, account for herbivore outbreaks, or stabilize competitive interactions in lower trophic levels. Therefore, their negative effects on the efficiency of biological control suggest that hyperparasitoids can affect the effectiveness of primary parasitoids (Poelman et al., 2022).

Baryscapus evonymellae (Bouché, 1834) (Hymenoptera, Eulophidae) is a faunistically well-known species that has been reported from Iran (Hesami et al., 2006). In the trophic chains, it was reported as a parasitoid of Lepidoptera and a hyperparasitoid of ichneumonid wasps (Noyes, 2019). Our recent sampling revealed the new aspects of the associated parasitoids and hyperparasitoids of *L. wiltshirei* in different localities of Iran updating the knowledge on the occurrence and biology of *B. evonymellae*.

MATERIAL AND METHODS

The collection was made from February to November 2022 from infested oak trees with *L. wiltshirei* in the Kohgiluyeh and Boyerahamd (Basht) and Fars (Rostam) provinces. The infested leaves were transferred to the Entomology Laboratory of Tabriz and Yasouj Universities and *L. wiltshirei* larvae were kept in plastic containers for the rearing of the parasitoids. In the laboratory, the mature larvae and pupae were placed individually in vials (10×8×5 cm) that were tightly sealed with cotton to maintain moisture for rearing. They were reared at 25±1ºC and checked daily to collect emerging adult parasitoids and hyperparasitoids. The remaining moth larvae and pupae were later dissected to determine if they were attacked by primary or hyperparasitoids. Obtained parasitoid and hyperparasitoid wasps were preserved in 75% ethanol for later taxonomic study. Dehydrated specimens were air-dried and stuck on a rectangular card for morphological study. An Olympus SZH stereomicroscope and a Keyence-5000 were used for morphological study and illustration of the specimens, respectively. The terminology for the morphological characters follows Graham's (1991) key to the genera and species used for determination. One hundred cocoons of priory identified braconid parasitoid, *Dolichogenidea. persica* were collected and separated from the infested larvae of *L. wiltshirei*. To determine the natural rate of hyperparasitism, each 10 cocoons was kept in a plastic box (8×7×4 cm) and transferred into a growth chamber [25±1ºC, 65±5 % R.H. and a photoperiod of 16:8 h (L:D)]. The boxes were daily checked and the number of hyperparasitoids that emerged was recorded. All material is deposited in the insect collection of the Hayk Mirzayans Insect Museum (HMIM), Iranian Institute of Plant Protection, Tehran, Iran.

RESULTS

Examination of reared specimens showed it belongs to the genus *Baryscapus* Förster, 1856 and consequently was identified under *Baryscapus evonymellae* (Bouché, 1834)

Taxonomic hierarchy

Order Hymenoptera Linneaus, 1758

Superfamily Chalcidoidea Latreille, 1817

Family Eulophidae Westwood, 1829

Genus *Baryscapus* Förster, 1856

Type species: *Baryscapus racemariae* (Ashmead, 1886)

***Baryscapus evonymellae* (Bouché, 1834)** (Figs 1–3)

Aprostocetus evonymellae (Bouché, 1834) Graham, 1961b:47; *Entedon cribrellae* Rondani, 1877a:174–175; *Eulophus evonymellae* Bouché, 1834:172; *Eutetrastichus evonimellae* (Bouché, 1834) Kalina, 1989:121; *Geniocerus evonymellae* (Bouché, 1834) Erdös, 1954:359; *Tetrastichus evonymellae* (Bouché, 1834) Walker, 1846:73.

Material examined. IRAN, Kohgiluyeh and Boyerahamd province, Basht, Lirab village (30°19'47"N, 51°31'11"E; 797m a.s.l.), 06.07.2023, A. Sedaratian-Jahromi leg.; Fars province, Rostam (30°25'14"N, 51°20'50"E), 19.05.2022, F. Ehteshami leg.

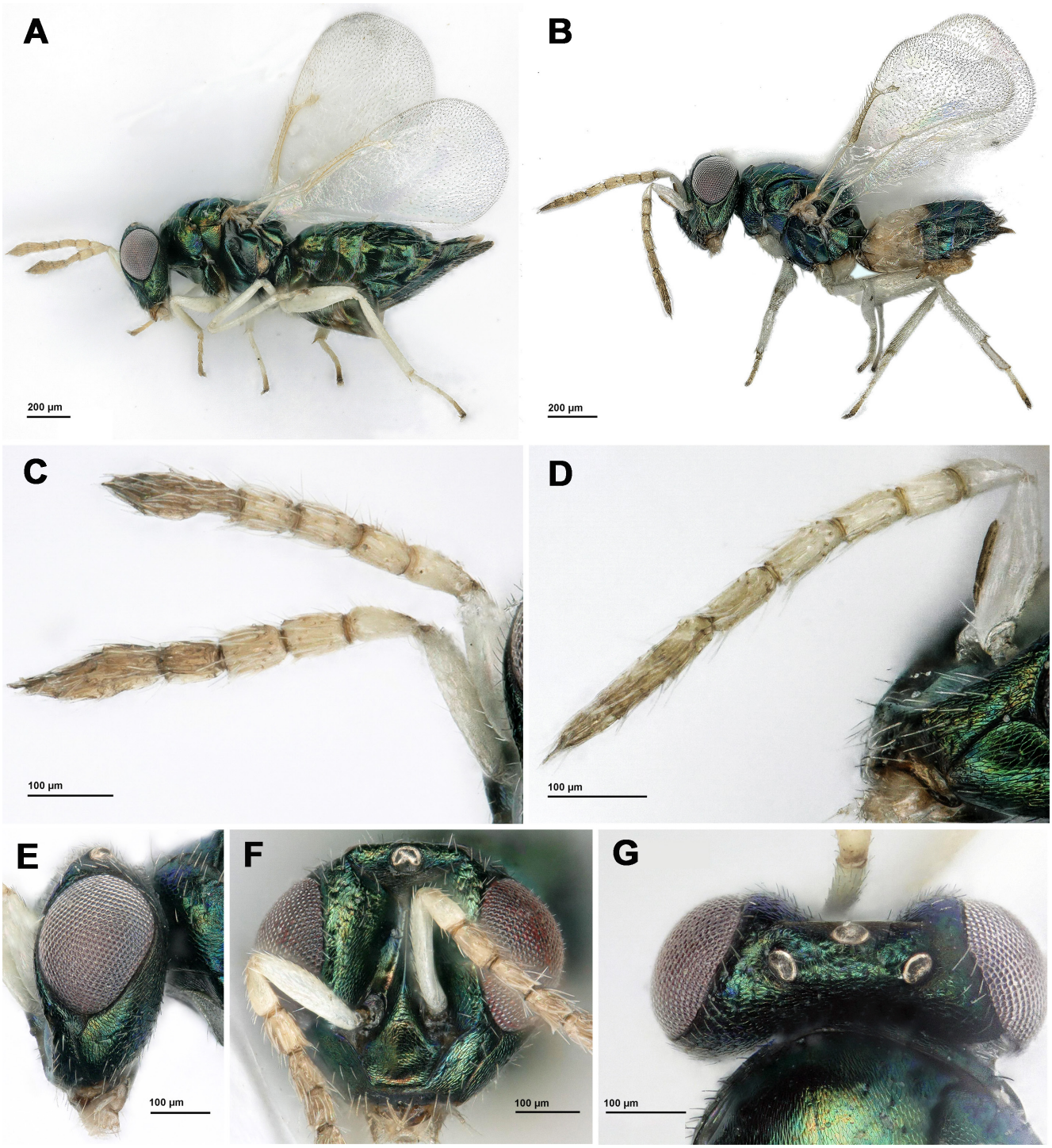

Figure 1. *Baryscapus evonymellae* (Bouché, 1834), habitus in lateral view. **A.** Female; **B.** Male. **C.** Female antenna; **D.** Male antenna; **E.** Head in lateral view; **F.** Head in frontal view; **G.** Head in dorsal view.

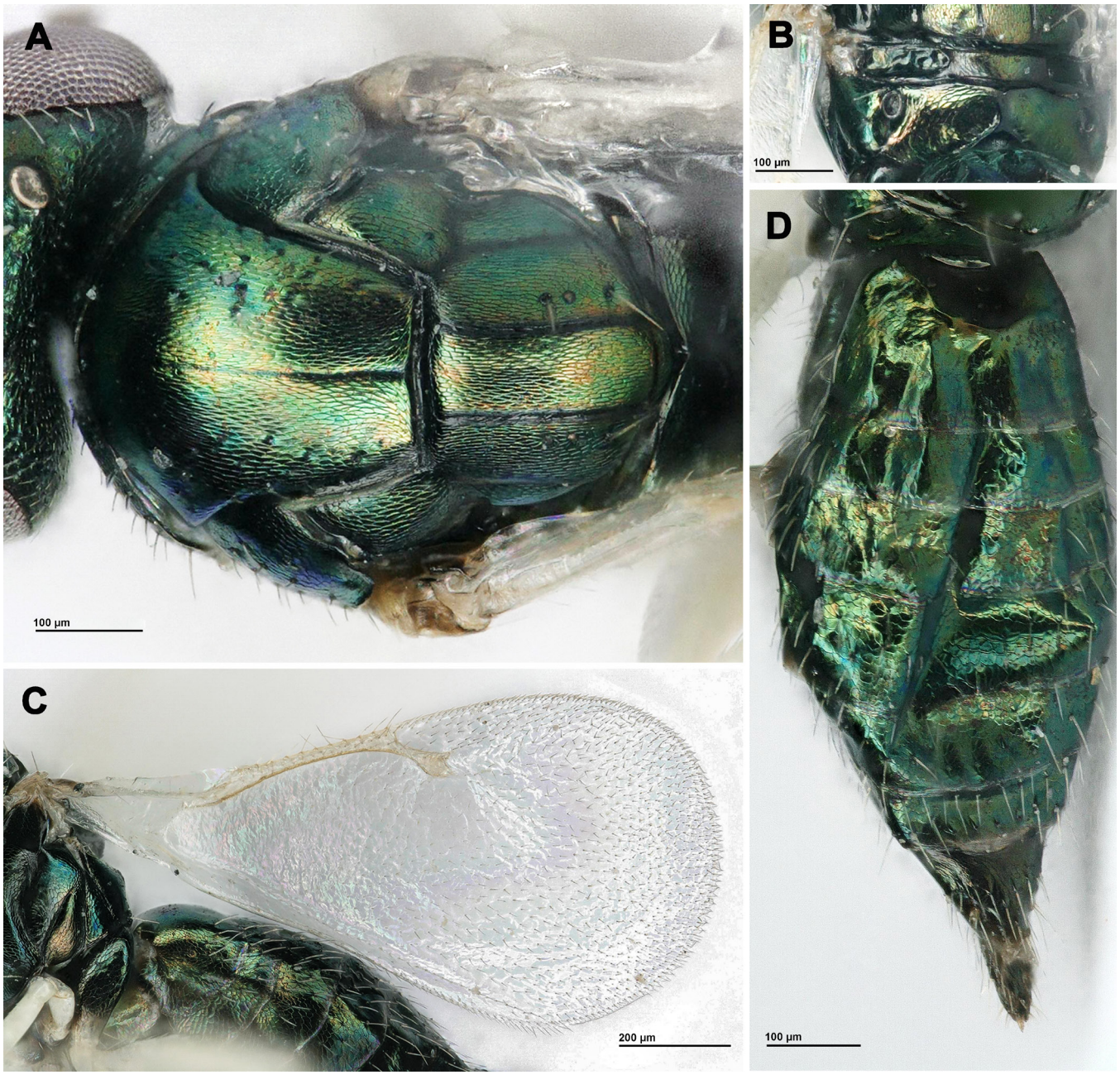

Figure 2. *Baryscapus evonymellae* (Bouché, 1834). **A.** Mesosoma in dorsal view; **B.** Propodeum in dorsal view; **C.** Fore wing; **D.** Metasoma in dorsal view.

***Main morphological characters*. Female.** Body length 1.4–1.8 mm (Fig. 1).

Coloration. Body metallic green with slight bluish or golden luster, scape entirely whitish yellow (Fig. 1C) [black, or partly yellow, rarely wholly yellow in European populations, Graham (1991)], pedicel and flagellum wholly yellowish-brown [yellow to brown in European populations, Graham (1991)], with articulations brownish; legs whitish-yellow, with coxae concolorous with mesosoma, trochanters whitish-yellow [femora black, distally yellow in European populations, Graham (1991)].

Diagnosis. Scape shorter than eye, not reaching median ocellus; pedicel + flagellum slightly longer than breath of mesoscutum; pedicel as long as first funicular, about 2 times as long as broad; all funiculars longer than broad; clava, about 2.7 times as long as broad, as long as second and third funiculars; clava with a terminal spine, about 0.35 times as long as third clavomere. Mid lob of mesoscutum finely

reticulate, with complete median line, 2–4 rows of setae laterally; mesoscutellum with submedian lines equidistant from each other and from sub-lateral lines (Fig. 2A); propodeum shiny, finely reticulate, with slightly raised median carina, about 0.3 times as long as mesoscutellum, medially as long as dorsellum (Fig. 2B). Marginal vein 2–2.8 times as long as stigmal vein (Fig. 2C). Metasoma lanceolate, about 2.3 times as long as broad, longer than head + mesosoma; hypopygium at mid-length of metasoma; ovipositor sheaths slightly projected (Fig. 2D).

Male. Similar to females except for the following characters (Fig. 1B): slightly smaller than female (1.3–1.7 mm); ventral plaque of scape brownish (Fig. 1D); legs wholly whitish-yellow (including coxae); metasoma with yellowish subbasal spot; scape with a ventral plaque, about half as long as scape; pedicel + flagellum about 1.3 times as long as breath of mesoscutum.

Biological data. We reared *B. evonymellae* from braconid cocoons, *Dolichogenidea persica* (Hymenoptera: Braconidae) and pupae of *Brachymeria tibialis* (Hymenoptera: Chalcididae). *Baryscapus evonymellae* is found to be a gregarious endoparasitoid of mature larvae and pupae of *D. persica*, *B. tibialis* (Figs 3C–E). Generally, 1–8 (average 3) wasps progeny develop from a *D. persica* cocoon and 1–72 (average 24) wasps from *B. tibialis* pupae, with a female: male ratio of 1.7: 1. The first emerging male wasp chews pupal body wall to exit and other emerging wasps in a brood often use the same hole but a second hole on the cocoon of the *D. persica* is also possible (Fig. 3H). The last instar larva and pupa of *B. evonymellae* hibernate inside the cocoon and pupal shield of the host and begin development around late May. The natural hyperparasitism rate of *B. evonymellae* on *D. persica* ranged between 9.1 and 81.8%, with an average of 40.9 and 65.0% in Kohgiluyeh-Boyerahmad and Fars provinces, respectively. The courtship and mating behavior of this species is similar to other chalcids (Fig. 3A) (Assem et al., 1980).

Distribution. This species is widely distributed in the Palaearctic region and it was reported from the USA and Sudan in the Nearctic and Afrotropical regions, respectively (Noyes, 2019) (Fig. 4).

DISCUSSION

Based on the available literature, this species was previously reported under *Tetrastichus* sp. (Mirzayans & Abai, 1974) and *Tetrastichus evonymellae* from Iran (Mozaffari et al., 1997). *Tetrastichus evonymellae* is a junior synonym of *B. evonymellae*, therefore, we hereby clarify the correct name and previously inaccurate reports. Our finding about the parasitoid-hyperparasitoid association confirms the reports of Mirzayans & Abai (1974) and Mozaffari et al. (1997) and approves the hyperparasitic activity of *B. evonymellae* on Lepidoptera. A wide host range was reported for *B. evonymellae* in Iran, including: *Yponomeuta* sp. (Lepidoptera: Yponomeutidae) (Shojaei, 1968; Graham, 1991; Hesami et al., 2006). Species of the families Cynipidae and Ichneumonidae (Hymenoptera), Lasiocampidae, Lyonetiidae and Pyralidae (Lepidoptera) were recorded as hosts of *B. evonymellae* (Noyes, 2019). In most rearing programs, all obtained parasitoids are generally considered primary parasitoids because the discrimination of tiny primary parasitoids from hyperparasitoids is very challenging. Poelman et al. (2022) mentioned highly specialized and skilled taxonomists as a critical necessity. Careful biological observation and dissection of host larvae and pupae can elucidate the biological association of parasitic wasps with their host. For example, Žikić et al. (2018) during an extensive sampling in Europe reported *B. evonymellae* as a primary of *Yponomeuta* spp. (Lepidoptera: Yponomeutidae), while at the same time the families Ichneumonidae and Braconidae with 53% and 10%, respectively, were the most abundant reared parasitoids.

A comprehensive examination may reveal its accurate association with *Yponomeuta* spp. as a primary parasitoid or hyperparasitoid via ichneumonid and braconid species. It seems that *B. evonymellae* has a high potential against biological control agents of *L. wiltshirei* to control its hosts considering its relatively high parasitism rate, successful development of a large number of wasps per single host, and a female-biased sex ratio. Sex ratio dynamics is relevant is an important factor in mass-rearing programs of parasitic wasps and their application as biological control (Werrern, 1984).

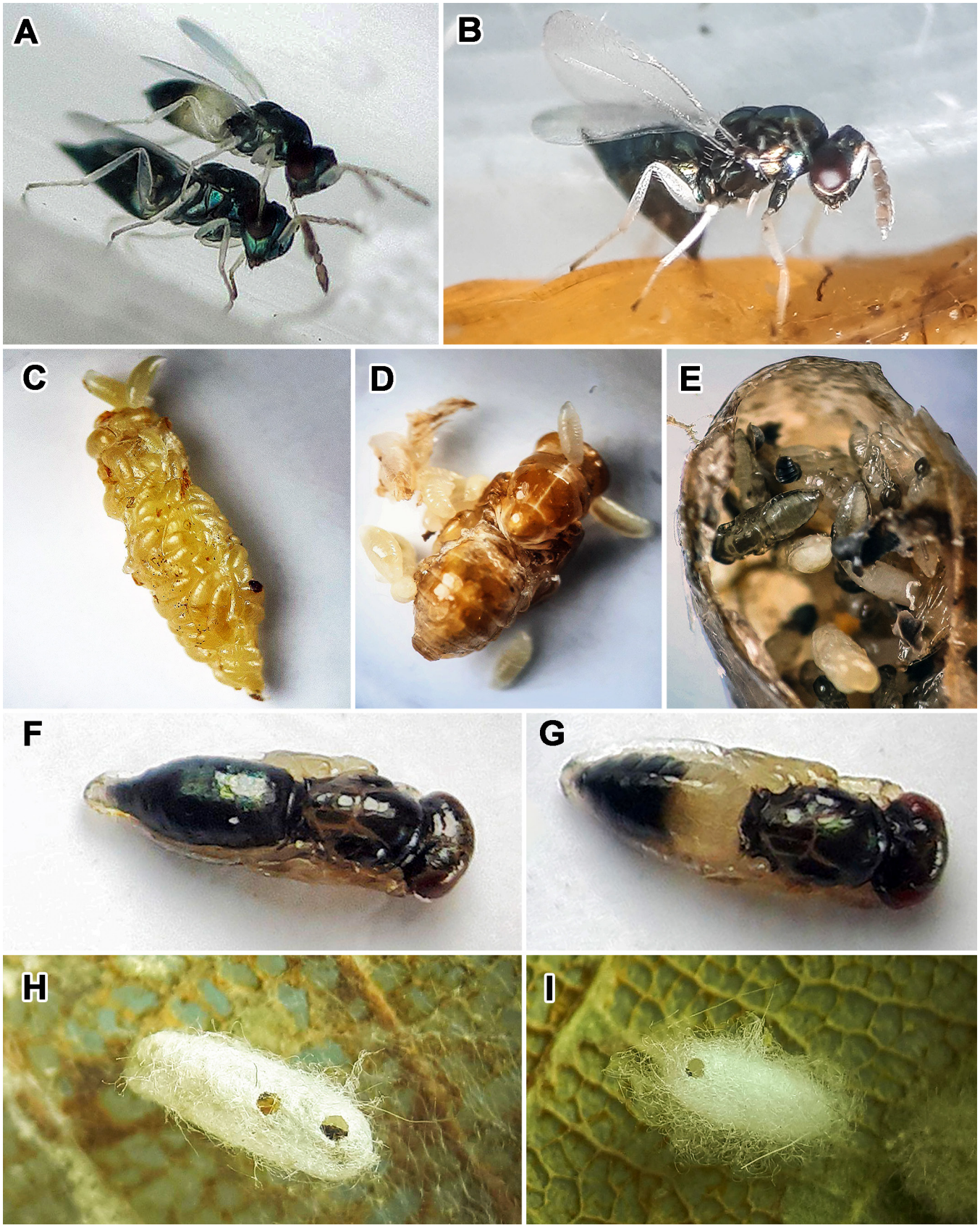

Figure 3. *Baryscapus evonymellae* (Bouché, 1834). **A.** Mating behavior in rearing box; **B.** Oviposition behavior of female; **C.** Larvae out of host; **D.** A pupa of *Brachymeria tibialis* with its hyperparasitoid larvae; **E.** Exuvia of host with pupae of hyperparasitoid; **F.** Pupa of female; **G.** Pupa of male; **H.** Cocoon of *Dolichogenidea persica* with two exit holes; **I.** Cocoon of *Dolichogenidea persica* with one exit hole.

Figure 4. World map indicating distribution pattern of *Baryscapus evonymellae* (Bouché, 1834).

László et al. (2016) believe parasitoid sex ratios are generally female-biased due to host size, density, and population sizes of parasitoids and reproductive parasites. These may well explain *L. wiltshirei* outbreak in the summer. On the other hand, in a food web, the top-down control of parasitoids by hyperparasitoids plays an important role in reducing competition between primary parasitoids (Poelman et al., 2022). For instance, in the studied case, *B. evonymellae* activity affects apparent competition between parasitoids, *Dolichogenidea persica* and *Brachymeria tibialis* associated with it. Poelman et al. (2022) have pointed out that their absence in the community may result in secondary extinctions of herbivores and their parasitoids.

These findings demonstrate the potential impact of *B. evonymellae* to interrupt the natural control of *L. wiltshirei,* highlighting the critical need for more detailed studies on its role in population fluctuations of the pest. By discovering the association between *B. evonymellae* and *D. persica*, *B. tibialis* mature larvae and young pupae as its potential hosts, we have to uncover key patterns, trends, and associations that help us to evaluate its possible contribution to outbreaks of *L. wiltshirei* in Zagros forests of Iran. Nenzén et al. (2018) proved the effect of hyperparasitoids on large-scale outbreaks of insect pests. According to Poelman et al. (2022), the negative effects of hyperparasitoids on the efficiency of biological control suggest that hyperparasitoids may be considered as balancing agents of selection on plant traits that enhance the effectiveness of primary parasitoids, especially where pests attack the reproductive organs of plants. Considering the vastness of the Zagros forests from the northwest to the south of Iran on the border of the Persian Gulf with an area of six million hectares, where oak, *Q. brantii* is the dominant species (Sagheb-Talebi et al., 2014; Moradi et al., 2021), the distribution of the pest and its parasitoids and hyperparasitoids complex is also conceivable. Therefore, this complex should always be under constant surveillance.

AUTHOR'S CONTRIBUTION

The authors confirm their contribution in the paper as follows: F. Ehteshami: the methodologist, data collector; M. Jafarlu: Data collector, taxonomic identification; H. Mohammadi: the methodologist, data collector; A. Sedaratian-Jahromi: Project supervisor, drafting, writing the introduction; S. Iranipour: Project supervisor, drafting, writing the methodology; H. Lotfalizadeh: The main researcher, conceptualization of work, project supervisor, taxonomic identification, writing the discussion; M. Kiany: the methodologist, data collector; The authors read and approved the final version of the manuscript.

FUNDING

This research received no specific grant from any funding agencies.

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.

ACKNOWLEDGMENTS

We thank Dr J.-Y. Rasplus (CBGP, Montferrier-le-lez, France) for providing facilities for taking photos of *Baryscapus evonymellae* in his laboratory.

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زنبور *Baryscapus evonymellae* (Bouché) (Hymenoptera: Eulophidae)، پارازیتویید ثانویهٔ پروانه سفید برگخوار بلوط *Leucoma wiltshirei* Collenette (Lepidoptera: Erebidae) در ایران

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| **تاریخ دریـافت:** ۲ آبان ۱۴۰۲ | **تاریخ پذیرش:** ۲۴ دی ۱۴۰۲ | **تاریخ انتشار:** ۲۹ دی ۱۴۰۲ |

چکیـــده: زنبور *Baryscapus evonymellae* (Bouché, 1834) متعلق به خانواده Eulophidae در بررسی‌های اخیر از روی پروانه برگخوارسفید بلوط *Leucoma wiltshirei* Collenette, 1938 از خانواده Erebidae، که توسط پارازیتوییدهای مختلف مورد حمله قرار گرفته بود، پرورش داده شد. این گونه تحت نام‌های مختلف از روی آفت فوق گزارش شده بود، از این‌رو در این تحقیق، شناسایی نامطمئن آن تصحیح و تثبیت شد. این زنبور از شفیره‌های دو گونه زنبور پارازیتویید مهم پروانه برگخوارسفید بلوط شامل *Brachymeria tibialis* Steffan, 1958 از خانواده Chalcididae و *Dolichogenidea persica* Abdoli, Mohammadi, Sedaratian-Jahromi & Farahani, 2023 از خانواده Braconidae پرورش داده شد. رابطهٔ پارازیتویید-میزبان این گونه با *D. persica* برای اولین بار ثبت می‌شود. مشخصات مورفولوژیک مهم زنبور هیپرپارازیتویید و برخی اطلاعات زیستی به همراه تصاویر آن ارایه و مورد بحث قرار گرفت.

واژگـــان کلیدی: براکنید، مهار زیستی، کالسید، جنگل، پارازیتویید، آفت، زاگرس