

## **New Record and DNA Barcoding of *Dolichogenidea laevigata* (Ratzeburg, 1848) as a Parasitoid of *Archips rosana* (Linnaeus, 1758) from Iran**

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### **ABSTRACT**

This is the first report for Iran of *Dolichogenidea laevigata* (Ratzeburg, 1848) (Hymenoptera: Braconidae: Microgastrinae) parasitizing larvae of the European leaf roller *Archips rosana* (Linnaeus, 1758) (Lepidoptera: Tortricidae). The parasitized larvae of *A. rosana* were obtained from different host plants. The occurrence of *D. laevigata* and its diagnosis are presented. In addition, a full DNA barcode (the mitochondrial cytochrome oxidase subunit I, (COI) barcoding region) of the species was obtained for the first time.

**Key words:** Microgastrinae, parasitized larvae, Lepidoptera defoliator, DNA barcoding.

## INTRODUCTION

The European leaf roller, *Archips rosana* (Linnaeus, 1758), is a defoliator of hardwood trees and shrubs that is distributed throughout Europe, North Africa, North America, Middle East, Iran, Iraq, Turkey, Azerbaijan and Kazakhstan (Ferriere, 1941; Kapidani & Duraj, 1991; Ulusoy, Vatansever, & Uygun, 1999; Güncan, Yoldaş, & Koçlu, 2013; Amano & Higo, 2015). The larvae of *A. rosana* roll and tie leaves together for shelter and feeding. This species is univoltine, and the adult moths fly from the end of May to the early of August with a peak flying period in the 2<sup>nd</sup> half of June. The larvae are polyphagous which attack fruit trees, forest trees and other trees of the family Rosaceae (Polat & Tozlu, 2010).

Biological control is one of the most effective and important components of integrated pest management programs used to suppress pests. Mayer & Beirne (1974) recorded 28 different parasitoid species attacking *A. rosana* larvae on apple trees. Polat & Tozlu (2010) collected 11 hymenopteran parasitoids of *A. rosana* belonging to Ichneumonidae, Pteromalidae, Chalcididae, Torymidae, Eulophidae and Eupelmidae. Aydoğdu (2014) was reported 22 hymenopteran parasitoids of *A. rosana* from cherry trees in Turkey, of which most of them were attributed to family Braconidae (13 species). Yu, van Achterberg, & Horstmann (2016) compiled all published information and listed 165 parasitoid species attacking *A. rosana* from different families of Hymenoptera, including 60 species of Braconidae.

Microgastrinae is one of the largest subfamilies of Braconidae, consisting of 81 genera and 2999 extant species worldwide (Fernandez-Triana, Shaw, Boudreault, Beaudin, & Broad, 2020). Until now, about 111 species of Microgastrinae have been documented for the fauna of Iran (Farahani, Talebi, van Achterberg, & Rakhshani, 2014; Ghafouri Moghaddam, Rakhshani, van Achterberg, & Mokhtari, 2018; Abdoli & Pourhaji, 2019; Abdoli, Talebi, & Farahani, 2019a; Abdoli, Talebi, Farahani, & Fernandez-Triana, 2019b; Abdoli, Talebi, Farahani, & Fernandez-Triana, 2019c; Zargar, Gupta, Talebi, & Farahani, 2019a, 2019b, 2020; Fernandez-Triana et al, 2020; Abdoli, Talebi, Fernandez-Triana, & Farahani, 2021).

The genus *Dolichogenidea* Viereck, 1911 is a large group with more than 360 described species in the worldwide, and more than 120 species have been found in the west Palaearctic region (Fernandez-Triana, Sakagami, & Shimizu, 2018; Liu, He, & Chen, 2018; Abdoli et al, 2019c; Fernandez-Triana et al, 2020). Recently, Abdoli et al (2019c) listed 13 species of *Dolichogenidea* and described *D. fernandeztrianai* Abdoli & Talebi, 2019 a as a new species from Iran. *Dolichogenidea laevigata* (Ratzeburg, 1848) is a solitary endoparasitoid wasp which is known to attack the caterpillars of Lepidoptera, especially the family Tortricidae (Yu et al, 2016). *Dolichogenidea laevigata* is distributed in the Palaearctic and Oriental regions (Yu et al, 2016).

The objective of this study is to improve our knowledge about biological control agents of *A. rosana* for its better management and we here introduce a new record of parasitoids from Iran as a part of the ongoing research on the systematic of Microgastrinae.

## MATERIAL AND METHODS

The specimens of the present study were collected in the National Botanical Garden of Iran in May 2019. The sampled site was located at 35 44' N, 51 10' E, elevation 1320m in Tehran, Iran. Larvae of *A. rosana* were collected from different host trees including *Acer* spp., *Quercus* spp., *Morus* sp., *Celtis* sp., *Parrotia persica*, where their leaves had been rolled by larvae (Figs. 1 A-B). Then, larvae were kept under laboratory conditions in plastic cages (Diameter: 17 and High: 25 cm) at 25±2°C, 16:8h (L: D) and reared on host-plant leaves to obtain adults for verification as *A. rosana*.

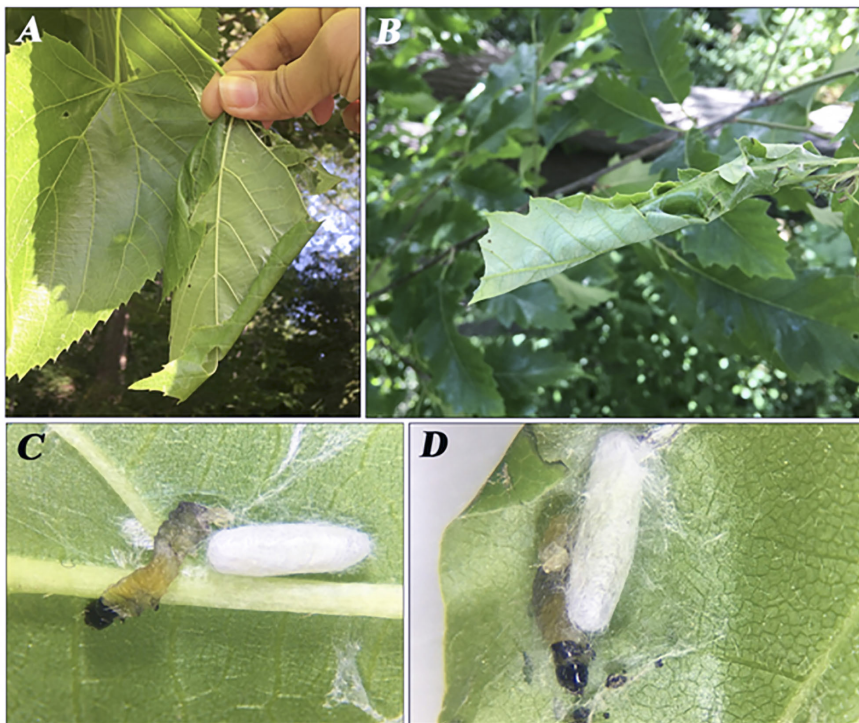


Fig. 1A-D. A-B, Damage of *Archips rosana* (rolled and tied leaves); C-D, Pupa of *Dolichogenidea laevigata* beside dead larva of *A. rosana*.

The parasitoid specimens were identified with the keys of Nixon (1972), Papp (1978), Tobias (1986), Chen & Song (2004) and Liu et al (2018). Morphological terminology follows Wharton, Marsh, & Sharkey (1997) for wing venation and Karlsson & Ronquist (2012) for the other body parts used in description of the new record. The abbreviations T1, T2, and T3 refer to the metasomal mediotergites 1, 2, and 3, respectively.

The specimens were photographed with a Keyence VHX-1000 Digital microscope, using a lens with a range of 13-130×. Multiple images through the focal plane later were combined to produce a single in-focus image. The software associated with

the Keyence system produced the focused images taken with that camera. The measurements were done using an Olympus™ SZX9 stereomicroscope equipped with a graticule. DNA barcoding of new record focused on the sequencing of a short standardized portion of the mitochondrial cytochrome c oxidase I gene (COI). The sample in this study has had legs removed for DNA extraction. DNA extraction method follows Brewster and Paoli (2013). The COI gene was amplified using primers (LCO1490-HC02198) following standard protocols (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). The barcode sequence was deposited in the National Center for Biotechnology Information (NCBI) and also, the sequence of the species was assigned in BOLD (Barcode of Life Datasystems).

The specimens of *D. laevigata* are deposited in the Collection of Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran.

## RESULTS

Pupae of wasps appeared by mid-late May beside dead larvae (Fig. 1 C-D). Only one species of Braconidae (e.g., *Dolichogenidea laevigata* (Ratzeburg, 1848)) is reported for the first time from Iran. In addition, mitochondrial cytochrome oxidase subunit I (COI) barcoding region has been sequenced for the first time.

### Taxonomy

#### ***Dolichogenidea laevigata* (Ratzeburg, 1848) (Figs. 2A-G)**

*Microgaster laevigatus* Ratzeburg, 1848, p. 50.

*Apanteles calcaratus* Ivaniv, 1899

*Microgaster hoplites* Ratzeburg, 1848

### Distribution in Iran

Tehran (This is a new record for Iran).

### General distribution

Oriental (China), Palaearctic (Armenia, Azerbaijan, Bulgaria, China, Former Czechoslovakia, Finland, France, Georgia, Germany, Hungary, Israel, Italy, Kazakhstan, Korea, Latvia, Lithuania, Moldova, Netherlands, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine, United kingdom, Uzbekistan, Former Yugoslavia) (Yu et al, 2016).

**Material examined:** Iran, Tehran province, National Botanical Garden of Iran, 15.05.2019, 6♂♂, 3♀♀. Host: *Archips rosana*. leg.: S. Farahani.

### Diagnosis (female)

Propodeum with areola weakly defined by an impression (Fig. 2E); in fore wing, vein R1 slightly longer than pterostigma; vein R1 4.30-4.50× as long as distance of

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vein R1 to vein 3RSb (Fig. 2F); T1 subparallel-sided (i.e., slightly narrower medially), weakly punctuate in posterior half and otherwise smooth, with a distinct protuberance on central area, length 1.50× posterior width; T2 smooth; T3 longer than T2 (Fig. 2G); hypopygium pointed apically and slightly protruding beyond apical tergites; ovipositor sheath with uniform width from base to apex and 1.50-1.60× metatibia (Fig. 2D); body black or dark brown; tegula, mouthparts and legs (except for coxae) yellow; pterostigma brown with a basal yellow spot; mesosoma in ventral view, with a pair of yellow bands.

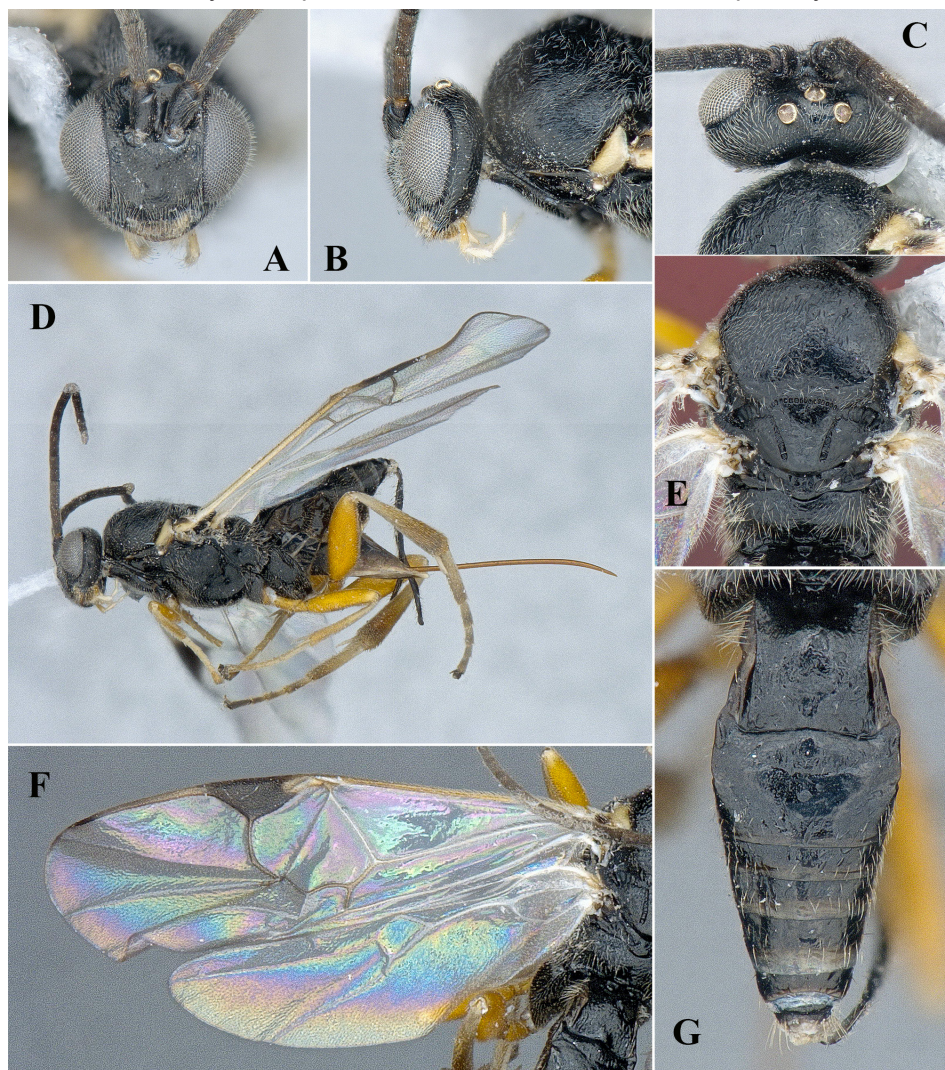


Fig. 2A-G. *Dolichogenidea laevigata*. A, Head, frontal view; B, Head, lateral view; C, Head, dorsal view; D, Habitus, lateral view; E, Mesosoma, dorsal view; F, Wings; G, Metasoma, dorsal view.

### Male

All characters similar to female, with the exception of T1 smooth, T1 length 2.00× its posterior width, T1 posterior half parallel-sided; antenna clearly longer than body (where as in female antenna as long as to slightly shorter than body); mesosoma clearly shorter than metasoma (where as in female mesosoma as long as metasoma).

### DNA barcode

The DNA barcode sequence for *D. laevigata* is available at the NCBI database (National Center for Biotechnology Information: <https://www.ncbi.nlm.nih.gov/>), with accession number MT180835.

The sequence of the species was assigned in BOLD (Barcode of Life Datasystems: <http://www.boldsystems.org/index.php>). The Barcode Index Number BOLD:AED8893, with the closest species found to be *Dolichogenidea phaloniae* (Wilkinson, 1940).

The COI sequence of *D. laevigata* is as below:

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ATATTTGGTTTATGATCTGGAATATTAGGATTTTCAATAAGATTAATTATTCGTT-
TAGAATTGGGAATACCTGGGAGGTTAATTGGTAATGATCAAATTTATAATAG-
TATAGTTACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTGTAATAATTG-
GAGGATTTGGTAATTGATTAATTCCTTTGATATTAGGATCACCTGATATATCATTTCC-
CTCGAATAAATAATAAGTTTTTGATTATTAATTCCTTCTTTATTTATATTAATTTAA-
GAGGATTTATTAATACTGGTGTGGAAACGGGTTGGACTGTTTATCCACCATTGT-
CATTAAATTTAGGTCATGGTGGAAATCAGTTGATTTAGGAATTTTTCTTTACATT-
TAGCTGGTGCTTCATCAATTATAGGTGCTGTTAATTTTATTACAACAATTTAAAT-
ATACGAACAAATTTATTTATAATAGATAAAAATATCTTTATTTTCTTGATCAGTTTTTAT-
TACTGCAATTTTATTATTATATCTTTACCTGTTTTAGCTGGAGCAATTACTATATTAT-
TAACTGATCGTAATTTAAATACAAGATTTTTTGGATCCTTCTGGTGGTGGAGATC-
CAATTTTATATCAACATTTATTTTGGTCAACCCAA.
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### Hosts

Yu et al (2016) listed all host records from the historical literature, but in many cases those records are incorrect (Fernandez-Triana et al, 2020).

## DISCUSSION

The genus *Dolichogenidea* is newly recorded from Tehran province. *Dolichogenidea laevigata* as a biological control agent is known to attack the forest Lepidopteran pest especially the family Tortricidae (Yu et al, 2016). In view of this finding, the potential of this parasitoid for biological control of *A. rosana* in Iran should be investigated and the information presented in this paper can be helpful in the development of biological control programs to manage of this pest.

This study has added one new record of *Dolichogenidea* from Iran, thus increase the total number of species to 15 in Iran (Abdoli et al, 2019a). *Dolichogenidea laevigata* is similar to *D. lineipes* from which it can be distinguished by the pale spot at the base of the stigma and the apical segments of the antenna are different (Nixon, 1972).

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The mitochondrial cytochrome oxidase subunit I (COI) of *D. laevigata* barcoding region was sequenced for the first time. There are almost 4,100 DNA-barcode compliant sequences of *Dolichogenidea* in BOLD representing 456 different BINs (Barcode Index Numbers), of these records, 239 species is presented.

## ACKNOWLEDGMENTS

This work is supported by Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran. We cordially thank section editor, Dr Jose Fernandez-Triana (Canadian National Collection of Insects, Ottawa, Canada), and an anonymous reviewer for their valuable comments and recommendations on the earlier version of this paper. Thanks to Department of Entomology, Tarbiat Modares University for providing facilities.

## REFERENCES

- Abdoli, P. & Pourhaji, A. (2019). Description of a new species of the genus *Pholetesor* Mason, 1981 and host association from Iran (Braconidae: Microgastrinae). *Redia*, 102, 55-60.
- Abdoli, P., Talebi, A.A., Fernandez-Triana, J., & Farahani, S. (2021). Taxonomic study of the genus *Microplitis* Förster, 1862 (Hymenoptera, Braconidae, Microgastrinae) from Iran. *European Journal of Taxonomy*, 744(1), 83-118.
- Abdoli, P., Talebi, A.A., & Farahani, S. (2019a). *Dolichogenidea fernandeztrianai* sp. nov. (Hymenoptera: Braconidae, Microgastrinae) from Iran. *Journal of Agricultural Science and Technology*, 21(3), 647-658.
- Abdoli, P., Talebi, A.A., Farahani, S., & Fernandez-Triana J. (2019b). *Venanides caspicus* sp. nov. from Iran, the first species of *Venanides* (Hymenoptera: Braconidae) described from the Palaearctic Region. *Acta Entomologica Musei Nationalis Pragae*, 59(3), 543-548.
- Abdoli, P., Talebi, A.A., Farahani, S., & Fernandez-Triana, J. (2019c). Three new species of the genus *Choerax* Mason, 1981 (Hymenoptera: Braconidae, Microgastrinae) from Iran. *Zootaxa*, 4545(1), 77-92.
- Amano, T. & Higo, Y. (2015). A convenient diagnostic polymerase chain reaction method for identifying codling moth *Cydia pomonella* (Lepidoptera: Tortricidae) among tortricid pests in cherries imported from western North America. *Applied Entomology and Zoology*, 50(4), 549-553.
- Aydođdu, M. (2014). Parasitoid abundance of *Archips rasana* (Linnaeus, 1758) (Lepidoptera: Tortricidae) in organic cherry orchards. *North-Western Journal of Zoology*, 10(1), 42-47.
- Brewster, J.D. & Paoli, G.C. (2013). DNA extraction protocol for rapid PCR detection of pathogenic bacteria. *Analytical Biochemistry*, 442(1), 107-109.
- Chen, J. & Song, D. (2004). Systematic studies on Microgastrinae of China (Hymenoptera: Braconidae). Fuchow, China: Fujian Scientific Publisher.
- Farahani, S., Talebi, A.A., van Achterberg, C., & Rakhshani, E. (2014). First record of *Microplitis rufiventris* Kokujev, 1914 (Braconidae: Microgastrinae) from Iran. *Check List*, 10(2), 441-444.
- Fernandez-Triana, J., Sakagami, K., & Shimizu, S. (2018). *Dolichogenidea maetoi* sp. nov. (Hymenoptera: Braconidae) from Japan, the first parasitoid wasp recorded from *Hyblaea fortissima* (Lepidoptera). *Acta Entomologica Musei Nationalis Pragae*, 58(1), 167-75.
- Fernandez-Triana, J., Shaw, M.R., Boudreault, C., Beaudin, M., & Broad, G.R. (2020). Annotated and illustrated world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). *ZooKeys*, 920, 1-1089.

- Ferriere, C. (1941). New Species of Euplectrini (Hym. Chaleidoidea) from Europe, Africa and Asia. *Bulletin of Entomological Research*, 32(1), 17-48.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5), 294-299.
- Ghafouri Moghaddam, M., Rakhshani, E., van Achterberg, C., & Mokhtari, A. (2018). A study of the Iranian species of *Choeras* Mason (Hymenoptera: Braconidae: Microgastrinae), with the description of a new species. *Zootaxa*, 4446(4), 455-476.
- Güncan, A., Yoldaş, Z., & Koçlu, T. (2013). Conservation and classical biological control of *Satsuma citruss* pests in western Turkey. International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palaearctic Regional Section, *Bulletin*, 95, 99-108.
- Kapidani, A. & Duraj, N. (1991). (Investigations on the biology and control of the tortricid *Archips rosana* L. in Albania). *Pflanzenschutzberichte*, 52(2), 79-88.
- Karlsson, D. & Ronquist, F. (2012). Skeletal morphology of *Opius dissitus* and *Biosteres carbonarius* (Hymenoptera: Braconidae), with a discussion of terminology. *Plos One*, 7(4), 1-38.
- Liu, Z., He, J., & Chen, X. (2018). The laevigata-group of the genus *Dolichogenidea* Mason, 1981 from China, with descriptions of 26 new species. *Zootaxa*, 4436(1), 001-074.
- Mayer, D.F. & Beirne, B.P. (1974). Aspects of the ecology of apple leaf rollers (Lepidoptera: Tortricidae) in the Okanagan Valley, British Columbia. *The Canadian Entomologist*, 106(4), 349-352.
- Nixon, G.E. (1972). A revision of the north-western European species of the laevigatus-group of *Apanteles* Förster (Hymenoptera, Braconidae). *Bulletin of Entomological Research*, 61, 701-743.
- Papp, J. (1978). A survey of the European species of *Apanteles* Först. (Hymenoptera, Braconidae: Microgasterinae), II. The laevigatus-group, 1. *Annales historico-naturales Musei nationalis hungarici*, 70, 265-301.
- Polat, A. & Tozlu, G. (2010). Investigations on the brief biology, host plants and parasitoids of *Archips rosana* (Linnaeus, 1758) (Lepidoptera: Tortricidae) in Erzurum. *Turkish Journal of Entomology*, 34(4), 529-542.
- Ratzeburg, J.T.C. (1848). *Die Ichneumoniden der Forstinsekten in entomologischer und forstlicher Beziehung*. Berlin.
- Telenga, N.A. (1955). Hymenoptera Vol. 5 No 4. Family Braconidae: Subfamily Microgasterinae, subfamily Agathidinae. *Fauna SSSR (NS)*, 61, 1-312.
- Tobias, V.I. (1986). *Subfamily Microgasterinae*. In G.S. Medvedev (Ed.). *Keys to the insects of the European part of the USSR. Volume III. Part IV* (pp. 1-883). Amerind Publishing Company.
- Ulusoy, M.R., Vatanserver, G., & Uygun, N. (1999). (The cherry pests, their natural enemies and observations on some important species in Ulukışla (Niğde) and Pozanti (Adana) province of Turkey). *Türkiye Entomoloji Dergisi*, 23(2), 111-120. (Turkish language)
- Wharton, R.A., Marsh, P.M., & Sharkey, M.J. (1997). *Manual of the New World genera of the family Braconidae (Hymenoptera)*. International Society of Hymenopterists.
- Yu, D.S., van Achterberg, C., & Horstmann, K. (2016). Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive. Nepean, Ontario, Canada.
- Zargar, M., Gupta, A., Talebi, A.A., & Farahani, S. (2019a). A review of the Iranian species of genus *Iconella* Mason (Hymenoptera: Braconidae: Microgastrinae) with description of two new species. *Zootaxa*, 4586 (3), 491-504.
- Zargar, M., Gupta, A., Talebi, A.A., & Farahani, S. (2019b). Three new species and two new records of the genus *Cotesia* Cameron (Hymenoptera: Braconidae) from Iran. *European Journal of Taxonomy*, 571, 1-25.
- Zargar, M., Gupta, A., Talebi, A.A., & Farahani, S. (2020). Description of a new species of the genus *Deuterixys* (Hymenoptera: Braconidae: Microgastrinae) from Iran. *Biologia*, 75, 267-272.