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Aphanogmus feltiellophagus sp. nov. (Hymenoptera: Ceraphronidae), a parasitoid of the acarivorous gall midge, Feltiella acarisuga Vallot (Diptera: Cecidomyiidae) in Iran

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ABSTRACT. During a laboratory biological study of a midge, *Feltiella acarisuga* (Vallot, 1827) (Diptera: Cecidomyiidae), acarivorous on two-spotted spider mites, some ceraphronid specimens were reared from the midge cocoons. A detailed morphological study and comparison with previously known species revealed that the ceraphronid is a new species of *Aphanogmus* Thomson, 1858 (Hymenoptera: Ceraphronidae). The new species is described as *A. feltiellophagus* Lotfalizadeh, **sp. nov.** and compared with closely related species. An identification key for all *Aphanogmus* species associated with predatory cecidomyiids is provided. As a parasitoid of the pupal stage of *Feltiella acarisuga*, the potential negative impact of *A. feltiellophagus* on the biological control of tetranychid mites is discussed.

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Keywords: acarivorous cecidomyiid, endoparasitoid, new species, spider mites

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INTRODUCTION

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Gall midges of the genus Feltiella Rübsaamen, 1910 (Diptera: Cecidomyiidae) are cosmopolitan species known as highly effective predators of tetranychid mites (Acari: Tetranychidae) (Gagné, 1995; Gagné & Jaschhof, 2021). Feltiella acarisuga (Vallot, 1827) is a well-known commercially available predator, whose larvae feed on tetranychid mites on various agricultural products (Barnes, 1933; Wardlow & Jobin, 1990). Based on multiple morphological studies, specimens of the Iranian acarivorous gall midges were identified as Feltiella acarisuga Vallot, but subsequent molecular studies demonstrated its similarity to F. tetranychi (Mollaei & Sadeghi-Namaghi, 2022). According to Gagné and Jaschhof (2021), there are two taxa named Feltiella tetranychi, one named by Rubsaamen that is regarded as a synonym of F. acarisuga and the other named by Kieffer which is mentioned as possibly synonymous with F. acarisuga. Our correspondence with international experts (Raymond J. Gagné, late Keith M. Harris, Netta Dorchin and Ximo Mengual) revealed that F. tetranychi Kieffer is an uncertain species, its status with F. acarisuga requires studying various populations worldwide, the DNA sequencing of more than one marker, and comparing the types of both species. Since the result of the molecular analysis was based on only one

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marker, the mitochondrial COI gene, and studying voucher specimens of *Feltiella tetranychi* was not possible, we relied on just the morphological description of the native *Feltiella* species and named it as *Feltiella acarisuga* based on the morphological identification.

Ceraphronidae consists of 400 species within 16 genera (Salden & Peters, 2023). Ceraphronids are distributed worldwide, and are known as parasitoids of Diptera, Hymenoptera, Lepidoptera, Coleoptera, Hemiptera, Neuroptera, Thysanoptera, and Trichoptera (Moser et al., 2024). These small endo- and ectoparasitic wasps are generally characterized by a black or brown body colour; no occipital depression; female antenna with 8 flagellomeres; axillar setae present; fore wing without pterostigma, a curved or straight stigmal vein; a wide-based mesoscutum anteriorly and with or without median mesoscutal sulcus; fore and mid tibial calcar simple at the apex and with a comb on the ventral face; mid tibia with one spur; Waterston's evaporatorium present; anterior part of synsternite simple; parossiculus and gonostipes fused (Masner & Dessart, 1967; Mikó & Deans, 2009). Aphanogmus Thomson, 1858 is known as a species-rich genus, approximately a quarter of them known as parasitoids or hyperparasitoids of various hosts in the orders Diptera (Cecidomyiidae) (Oatman, 1985; Gilkeson et al., 1993; Matsuo et al., 2016), Hymenoptera (Bethylidae, Ichneumonidae, Braconidae) (Polaszek & LaSalle, 1995), Lepidoptera (Gelechiidae, Noctuidae, Pyralidae) (Polaszek & Dessart, 1996; Youssef et al., 2022), Coleoptera (Cybocephalidae) (Evans et al., 2005), Thysanoptera (Thripidae) (Dessart & Bournier, 1971), Neuroptera and Trichoptera (Moser et al., 2024). The genus comprises 140 species globally (Johnson & Musetti, 2004; Buhl et al., 2010; Matsuo et al., 2016). It is separated into three species groups: clavicornis group, tenuicornis group and fumipennis group (Evans et al., 2005).

Aphanogmus floridanus Ashmead and A. fulmeki Szelényi are known as endoparasitoids of the cecidomyiids Feltiella acarivora Zehntner (Oatman, 1985); and F. acarisuga, F. acarivora, Aphidoletes aphidimyza Rondani and Mycodiplosis sp. (Diptera: Cecidomyiidae) (Dessart, 1992), respectively. Most recently two new Aphanogmus species associated with cecidomyiid mite predators were described: Aphanogmus kretschmanni Moser and A. cecidovorus Ranjith from the Palaearctic and Oriental regions respectively (Moser et al., 2023; Ranjith et al., 2023). A few sporadic records of the superfamily Ceraphronoidea, were documented from Iran (Rakhshani et al., 2005, 2010; Rezaei et al., 2006; Darsoei et al., 2011; Jafari Ahmadabadi & Modarres Awal, 2012; Atbaei et al., 2018). A literature review shows that there is no report of the occurrence of the family Ceraphronidae in Iran.

This study aims to report the family's first finding in Iran and describe the new species of *Aphanogmus* that was reared from *Feltiella acarisuga*.

MATERIAL AND METHODS

Native predatory gall midge, Feltiella acarisuga (Vallot) larvae and pupae were collected periodically during May-August (2018) from spider mite colonies on various host plants (Urtica dioica L., Lactuca scariola L. and Rubus sp.) in the countryside around Mashhad, Northeastern Iran. Larvae and pupae were transferred into Petri dishes in a growth chamber (LD 16:8, 21±1°C, RH 75±5%) and monitored until gall midges and/or possible parasitoids emerged. Emerged adult parasitoids were then preserved in 75% ethanol for the morphological examinations. Reared parasitoids were identified based on available keys and descriptions (Szelényi, 1940; Dessart, 1963; Alekseev, 1988; Buhl et al., 2010; Moser et al., 2023; Ranjith et al., 2023). Air-dried mounted specimens were examined with a Leica® M205C stereomicroscope and photographs were taken using a Sony® a7R III camera (Sony Group Corporation, Tokyo, Japan) connected to a 10× Mitutoyo Plan Apo Infinity Corrected Objective using a 16 mm tubular adaptor stand on a motorized Auto-focus macrophotography stage (Omega Optics Co. Ltd., Iran). The series of 100-120 partially focused images were stacked into a single in-focus image using Helicon Focus® v. 7 (Helicon Soft Ltd., Kharkiv, Ukraine). The light source was constructed using the twinn 9 Volt White High Power LED SMD Bead providing a smooth and diffused light covering all aspects of the specimens. Stacked photos were retouched using Adobe® Photoshop CS6. Adult morphological terminology follows Mikó and Deans (2009). The terminology for sculpture was based on the definitions provided by Harris (1979). The holotype and paratypes are deposited in the Hayk Mirzayans Insect

Museum, Iranian Institute of Plant Protection, Tehran, Iran (HMIM).

Abbreviations of morphological characters: $\mathbf{fl_1}$ - $\mathbf{fl_n}$, flagellomeres 1–n; LOL, lateral ocellar line (= minimum distance between the anterior and a posterior ocellus); \mathbf{mv} , marginal vein; \mathbf{OD} , maximum ocellar diameter; \mathbf{OOL} , ocello-ocular line (= distance between the posterior ocellus and the eye); \mathbf{POL} , distance between posterior ocelli; \mathbf{stv} , stigmal vein.

RESULTS

An adult male and six adult female ceraphronids emerged from the collected pupae. The emerged specimens were different from all other known ceraphronids and the species is described as *Aphanogmus feltiellophagus* Lotfalizadeh **sp. nov.** The new species seems to be a pupal endoparasitoid of *Feltiella acarisuga* Vallot.

Taxonomic hierarchy

Class Insecta Linnaeus, 1758

Order Hymenoptera Linnaeus, 1758

Family Ceraphronidae Haliday, 1833

Genus Aphanogmus Thomson, 1858

Type species. *Aphanogmus fumipennis* Thomson, 1858.

Aphanogmus feltiellophagus Lotfalizadeh, sp. nov. (Fig. 1A-F)

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Material examined. **Holotype** ♀: Iran, Khorasan Razavi province, Azghad (36.2005°N, 59.3362°E), July-August 2018, ex *Feltiella acarisuga* on *Urtica dioica*, M. Mollaei leg., deposited in HMIM. **Paratypes**: 6♀♀ & 1♂, same data as holotype, deposited in HMIM.

Type materials conditions. All type materials were card-mounted (3 females on rectangular cards and the rest on card points). All of these specimens are in good condition except two females that miss flagemomeres, one female misses the head and one of the females is mounted with dissected head, mesosoma and metasoma.

Etymology. The species is named after the host generic name, *Feltiella acarisuga* (Diptera: Cecidomyiidae).

Diagnosis. Morphologically, *A. feltiellophagus* Lotfalizadeh, **sp. nov.** falls into the *tenuicornis* species group based on the absence of a median mesoscutal sulcus, and the presence of a metasomal basal carina. However, among members of this group, the new species can be distinguished by the key to species provided below in combination with the following characters: scape 0.6 times as long as height of head; pedicel 1.7 times as long as fl₁; fl₇ 1.9 times as wide as fl₁; club of antenna 1 segmented; fore wing hyaline; stv 0.75 times as long as mv; relative length of POL:OD 2; relative length of OOL:OD 1.6. According to a key to the Palaearctic species of *Aphanogmus* (Szelényi, 1940), the new species runs to *A. gracilicornis* Förster based on the following characters: Frons transverse; antenna clavate, club 1 segmented; stv present and at least as long as the mv. However, the new species could be distinguished from *A. gracilicornis* with clavate antenna while *A. feltiellophagus* Lotfalizadeh, **sp. nov.** has non-clavate antenna with cylindrical funiculars.

Description. — Female (Fig. 1A). Body length 0.82 (0.76–0.84, n = 7) mm. Head dark brown. Scape yellow; pedicel and all flagellomeres yellowish brown. Mesosoma dark brown. Fore wing hyaline. All coxae dark brown basally and yellow in apical half; all femora yellow-brown; all tibiae yellow; tarsi pale yellow. Metasoma brown.

Head. Head superficially reticulated, in frontal view (Fig. 1C) 1.1 times as wide as high, in dorsal view (Fig. 1F) 1.7 times as wide as long, 1.1 times as wide as mesosoma; interocular distance 0.6 times as long as head width; malar space 0.6 times as long as eye height; lateral margin of torulus distinctly raised;

intertorular carina distinct; frontal depression smooth; ocellar foveae absent; preocellar pit absent; facial pit absent; preoccipital furrow present and extends from anterior ocellus to occipital foramen; preoccipital carina absent; preoccipital lunula absent; occipital carina present; occipital depression absent; occipit smooth; POL 2 times as long as OD, OOL 1.6 times as long as OD, LOL shorter than OD (0.8 as long as OD). Antenna (Fig. 1D) with 10 antenomeres; scape 0.6 times as long as height of head, 0.9 times as long as distance between inner orbits; pedicel 1.7 times as long as fl_1 ; the following segments gradually widened; fl_1 1.7, fl_2 1.1, fl_3 1.1, fl_4 0.9, fl_5 0.7, fl_6 1.1, fl_7 1.0 and fl_8 2.0 times as long as wide; fl_7 about as long as fl_1 .

Mesosoma. Mesosoma 1.4 times as long as wide; about as long as high; mesoscutum reticulate, sparsely setose (Fig. 1F); setal base slightly pustulate; median mesoscutal sulcus absent; notaulus absent; parapsidal line absent; interaxillar sulcus present; scutoscutellar sulcus angled medially, foveolate, continuous with interaxillar sulcus; dorsal axillar area and mesoscutellum sculptured as mesoscutum; mesoscutellum 1.1 times as long as wide; anterior mesopleural sulcus distinct; anterior mesopleural area finely reticulate with several setae; dorsal mesometapleural carina straight; anterior mesopleural sulcus perpendicularly intersecting dorsal mesometapleural carina; metapleural carina distinct, extends near dorsal mesometapleural carina. Metacoxa bare dorsally; longitudinal metacoxal carina present at base. Fore wing (Fig. 1F) 2.6 times as long as wide, hyaline; stv 0.75 times as long as mv.

Metasoma. Syntergum with distinct transverse carina anteriorly, as long as wide, smooth, occupying 0.5 times total length of metasoma; syntergum with short longitudinal striae.

MALE (Fig. 1B). Differs from female as follows: Body length 0.72 mm; Antenna (Fig. 1E) with 11 antennomeres; flagellomeres pale yellow, longer than wide (at least 2 times as long as wide), flagellar setae long, 2.0 times width of flagellomeres; legs mainly brownish.

Distribution. Northeastern Iran.

Host. Feltiella acarisuga (Vallot) (Diptera: Cecidomyiidae). From each host cocoon, one adult parasitoid emerged.

Key to species groups and species of *Aphanogmus* associated with acarivorous Cecidomyiidae (Diptera)

| 1 | Median mesoscutal sulcus present fumipennis group |
|---|--|
| | Note. in this group <i>A. flavigastris</i> Matsuo, 2016 was originally described from Japan as a parasitoid of <i>Feltiella acarisuga</i> (Vallot) and <i>F. acarivora</i> (Zehntner) it is characterised by scape about 0.6 times as long as height of head, pedicel 2.0–2.5 times as long as fu ₁ , fu ₇ about 2.0 times as wide as fu1; POL about 3.5 times OD, OOL about 2 times OD; metasoma with basal carina present; fore wing with a darkly pigmented band, stigmal vein 1.4–1.5 times as long as marginal vein. |
| _ | Median mesoscutal sulcus absent |
| 2 | Note. in this group <i>A. fulmeki</i> Szelényi was reported as a parasitoid of <i>F. acarisuga, F. acarivora, Aphidoletes aphidimyza</i> (Rondani), and <i>Mycodiplosis</i> sp. and its diagnostic characters are as follow: scape about 0.6 times as long as height of head, pedicel 2.1 times as long as fu ₁ , fu ₇ about 1.8 times as wide as fu ₁ ; POL about 2.1 times OD, OOL about 2.3 times OD; metasoma without basal carina; fore wing hyaline, stigmal vein 1.5 times as long as marginal vein. |
| _ | Metasomal basal transverse carina present |
| 3 | Fore wing hyaline, without dark band |
| 4 | Transverse band on the fore wing extends from subbasal to the basal half of the stigmal vein; stigmal vein distinctly curved, 1.8 times as long as the marginal vein; metasoma 1.4 times as long as mesosoma A. cecidovorus Ranjith, 2023 |
| _ | Transverse band present before marginal vein; stigmal vein straight, 1.5 times as long as the marginal vein; metasoma not longer than mesosoma |

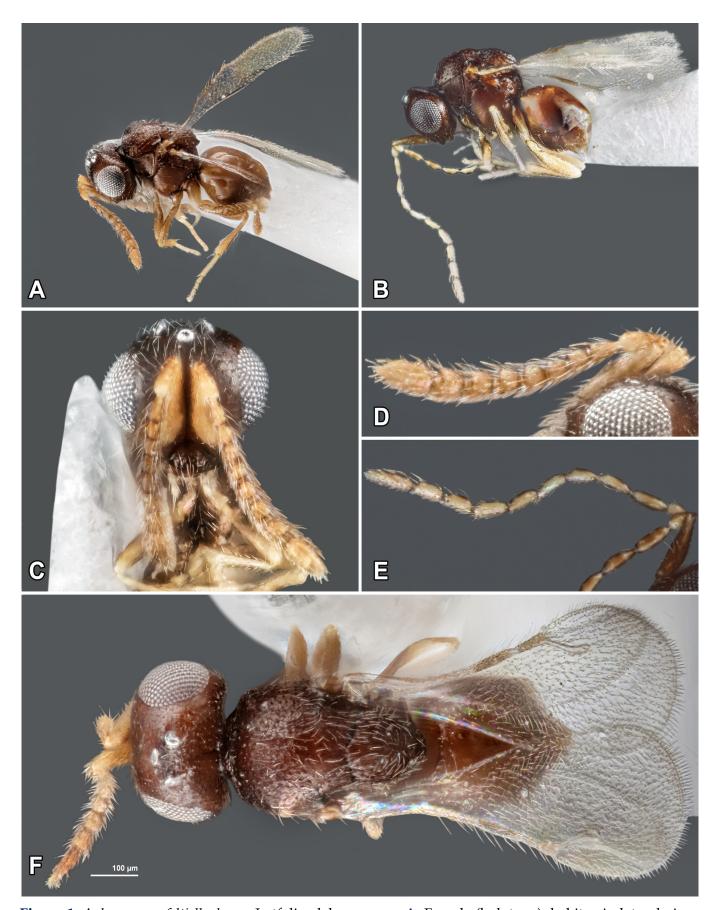


Figure 1. *Aphanogmus feltiellophagus* Lotfalizadeh, **sp. nov. A.** Female (holotype), habitus in lateral view; **B.** Male (paratype), habitus in lateral view; **C.** Head and antennae of female (holotype) in frontal view; **D.** Antenna of female (holotype); **E.** Antenna of male; **F.** Female (paratype) habitus in dorsal view.

DISCUSSION

This is the first record of the family Ceraphronidae from Iran. Although there have been no prior reports or findings regarding this particular family in the country, it is noteworthy that Ceraphronidae species have been identified in neighbouring countries, including Turkey (Topakci, 2022), Syria (Youssef et al., 2022), Pakistan (Polaszek & LaSalle, 1995) and Azerbaijan (Maharramova, 2010). *Aphanogmus* species are categorized into three distinct groups: *clavicornis*, *tenuicornis*, and *fumipennis* based on a median sulcus on the mesoscutum and a basal carina on the metasoma (Evans et al., 2005). The new species, *Aphanogmus feltiellophagus* belongs to the *tenuicornis*-group due to the absence of a median mesoscutal sulcus, and the presence of a metasomal basal carina.

The biological association of *Aphanogmus* species with gall midges (though they are not actual gall inducers) of the family Cecidomyiidae has been reported (Bakke, 1955; Laborius, 1972; Matsuo et al., 2016; Ranjith et al., 2023). Species of *Aphanogmus* which have an association with acarivorus cecidomyiids include: *A. fulmeki* Szelényi from the *clavicornis*-group and *A. flavigastris* Matsuo from the *fumipennis*-group. Both are reported as parasitoids of *Feltiella acarisuga* and *F. acarivora*. *Aphanogmus floridanus* Ashmead and *A. cecidovorus* Ranjith from the *tenuicornis*-group are reported as parasitoids of *Feltiella acarivora* and *Microdiplosis pongamiae* Mani, respectively (Oatman, 1985; Dessart, 1992, Matsuo et al., 2016, Ranjith et al., 2023). The newly described species is an endoparasitoid of the cecidomyiid, *Feltiella acarisuga*, hatching in the pupal stage. This is the first record of a parasitoid of acarivorus cecidomyiids in Iran. *Aphanogmus* species appear to parasitize one of two categories of hosts: those that are weakly concealed and exhibit considerable activity, or those that are well-concealed and relatively inactive (Moser et al., 2023). The new *Aphanogmus* species seems to parasitize *Feltiella acarisuga* pupae which are almost exposed and not concealed in galls. Moser et al. (2023) believe gall midges are often quite active and weakly-concealed hosts in the galls attacked by *Aphanogmus* species.

In this study, the identification of *Feltiella acarisuga* was solely based on morphological characteristics as no voucher specimens were available for molecular analysis. Nevertheless, prior research has demonstrated the effectiveness of morphological traits in classifying Cecidomyiidae within a broader phylogenetic framework (Dorchin et al., 2019). On the other hand considering the limitations of using cytochrome oxidase subunit I (COI) for distinguishing closely related species (Zaidi et al., 2011; Dai et al., 2012) emphasize the need for multiple gene markers in molecular analysis. Subsequent studies are needed to clarify the identity of *Feltiella acarisuga* comparing *F. tetranychi*.

Since the *Aphanogmus* species act as a negative agent in controlling phytophagous mites by *Feltiella* species, seasonal monitoring of its abundance and potential negative effects on the biological control of tetranychid mites need to be evaluated because it can affect the successful application of *Feltiella* species in the studied area.

AUTHOR'S CONTRIBUTION

The authors confirm their contribution to the paper as follows: M. Mollaei & H. Sadeghi-Namaghi: Fieldwork, collecting the specimens and writing the manuscript; H. Loftalizadeh: Identification of specimens and writing the manuscript; F. Minab: photography and writing the manuscript. All authors 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 HMIM and are available from the curator, upon request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study only included plants and arthropod material, and all required ethical guidelines for the treatment and use of animals were strictly adhered to in accordance with international, national, and institutional regulations. No human participants were involved in any studies conducted by the authors for this article.

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 .(Hymenoptera: Ceraphronidae) Aphanogmus feltiellophagus sp. nov) پارازیتویید پشهٔ گالزای کنهخوار Diptera: Cecidomyiidae) Feltiella acarisuga Vallot) در ایران

مائده ملائي الله حسين صادقي نامقي المقي عسين لطفعلي زاده و فرزاد ميناب الم

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چکیده: طی بررسی آزمایشگاهی پشه گالزای کنهخوار، (Vallot, 1827) از خانواده Ceraphronidae روی کنه تارتن دونقطهای، تعدادی زنبور پارازیتویید از خانواده شد. مطالعه دقیق ریختشناسی و مقایسه آنها با گونههای شناختهشده، نشان داد این زنبور پارازیتویید، گونهای جدید از جنس Hymenoptera: Ceraphronidae) Aphanogmus Thomson است. گونه پارازیتویید، گونهای جدید با نام .feltiellophagus Lotfalizadeh, sp. nov. توصیف و با گونههای نزدیک به خود مقایسه شد. کلید شناسایی تمام گونههای علام هستند، تهیه شد. اثرات منفی بالقوه این گونه به عنوان پارازیتویید مرحله شفیرگی پشه شکارگر کنهها مرتبط هستند، تهیه شد. اثرات منفی تارتن مختصرا مورد بحث قرار گرفت.

واژگان کلیدی: سسیدومید کنهخوار، اندوپارازیتویید، گونه جدید، کنههای تارتن