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Review of the Indian Species of *Evania* (Hymenoptera: Evanioidea: Evaniidae)

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ABSTRACT

The Indian species of *Evania* Fabricius (Hymenoptera: Evaniidae) are revised. Only two species, *E. mukerjii* Mani, 1943 and the widespread species *E. appendigaster* (L., 1758), are valid. Eight species, namely, *Evania abrahami* Joseph, *E. agraensis* Joseph, *E. johni* Joseph, *E. nurseana* Cameron, *E. rubrofasciata* Brues, *E. simlaensis* Cameron, *E. trivandrensis* Joseph, and *E. unipunctata* Joseph, are synonymised under *E. appendigaster.* Redescribed the two recognized Indian species of *Evania, E. appendigaster and E. mukerjii*, with distribution and host data provided.

Key words: Synonymy, ensign wasps, hatchet wasps, India, distribution.

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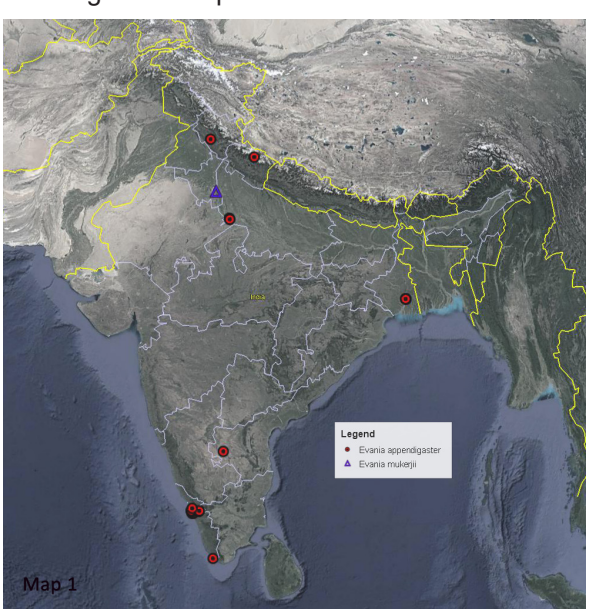
INTRODUCTION

Evaniidae (Hymenoptera), also known as ensign wasps or hatchet wasps, are most diverse in the tropics while found in relatively fewer numbers in temperate regions (Basibuyuk, Rasnitsyn, Fitton, & Quicke, 2002; Deans, 2005). Evaniids are primary parasitoids of cockroaches, and they lay their eggs on cockroach oothecae so that their larvae emerge as a solitary predator of cockroaches. In India, the family Evaniidae is represented by six genera, viz. *Evania* Fabricius (02 species), *Parevania* Kieffer (03 species), *Prosevania* Kieffer (17 species), *Vernevania* Huben & Deans (01 species), *Zeuxevania* Kieffer (06 species) and *Brachygaster* Leach (02 species). This study examines the genus *Evania* in India, which comprised ten species before this study (Kumar, Gopi, & Rajmohana, 2017). *On the other hand, Evania appendigaster* (L.) is a common and widely distributed species (Deans, 2005) and shows a high degree of variation in morphology. For this reason, many species have been described from different regions based on different morphotypes. Deans (2005) indicated many species of *Evania* are probable synonyms of *E. appendigaster*. However, based on the critical examinations of all the type specimens of Indian *Evania* and the specimens from National Zoological Collections, Zoological Survey of India, Kolkata, confirmed that only two species (*E. appendigaster* and *E. mukerjii*) are valid (Map 1). Remaining species, viz., *E. abrahami* Joseph, *E. agraensis* Joseph, *E. johni* Joseph, *E. nurseana* Cameron, *E. rubrofasciata* Brues, *E. simlaensis* Cameron, *E. trivandrensis* Joseph, *E. unipunctata* Joseph are synonymised with *E. appendigaster* (Linn.). Even though *E. appendigaster* is a widespread common species, and the available descriptions are not sufficient to recognize the species.



Map 1. Map showing distribution of *Evania* species in India.

MATERIALS AND METHODS

The present study was based on exiting and freshly collecting specimens in the Hymenoptera section, Zoological Survey of India, Kolkata. Specimens were collected by net sweeping, killed in ethyl acetate, and stored in 70% ethyl alcohol. They were later dried and pinned. Specimens were studied using a Leica M205A stereo zoom microscope and photographs and relative measurements (in mm.) were taken using a Nikon SMZ 25. The genus is identified using the key provided by Mani & Muzaffer (1943), Deans & Kawada (2008). Voucher specimens are deposited in the National Zoological Collections of Zoological Survey of India, Kolkata, India. The terminology is followed after Crosskey (1951) and Kawada (2011).

The following abbreviations are used in the text: F - Female; M - Male; F1-13 - Funicle segments 1-13; OOL - Minimum distance between the posterior ocellus and eye margin; POL - Minimum distance between the two posterior ocelli; AOL - Minimum distance between the posterior ocellus and anterior ocellus; BMNH - The Natural History Museum, London, United Kingdom; IARI - Division of Entomology, Indian Agriculture Research Institute, Pusa, New Delhi, India; LSUK - The Linnaean Society of London, Burlington House, Piccadilly, London, United Kingdom; MNHN - Museum National d'Histoire Naturelle, Paris, France.

RESULTS

Taxonomy

Evania Fabricius, 1775

Evania Fabricius, 1775: 345. Type species: *Sphex appendigaster* (Linnaeus), by designation of Latreille, 1810: 297.

Diagnosis. Head in frontal view wider than long and in lateral view slightly compressed; clypeus convex; mandibles bidentate; median ridge between antennal insertion extending almost to anterior ocellus; antennal sockets within upper third of head; fore wing extending beyond petiole, with six cells enclosed by tubular or nebular veins, and fore wing vein RS+M present, separating 1st submarginal and 1st subdiscal cells; distance between fore and mid coxae nearly equal to distance between mid and hind coxae; female metasoma in lateral view triangular, metasomal tergite VIII expanded dorsally (Deans & Huben, 2003)

Hosts. Species of *Evania* are primary parasitoids of cockroach ootheca (Blattidae: Blattinae) (Roth, 1989; Westwood, 1843; Lit, 1988; Yeh & Mu, 1994; Fox et al, 2012). Although with this revision only two species of the genus are now recognized from India, the hosts of only one species, *E. appendigaster*, are known.

Species and distribution. The genus *Evania* is worldwide in distribution, with 27 valid species known plus 32 names that are *incertae sedis*. The distribution of valid species and *incertae sedis* (in brackets) is as follows: Oriental, 11 (10); Palearctic, 7 (3); Afrotropical, 2 (1); Ethiopian, 6 (1); Neotropical, 4 (12); Australian, 2 (3); and Nearctic,

1 (0). At least two species, *E. appendigaster* and *E. dimidiate* Spinola are known from more than one zoogeographical region (Deans, Yoder, & Dole, 2020; Jennings, 2020).

***Evania appendigaster* (Linnaeus)** (Fig. 1)

Ichneumon appendigaster Linnaeus, 1758: 566 [type lost, LSUK]. Dalla Torre 1902: 1076, catalog. Townes 1949: 527- 528].

Sphex appendigaster Linnaeus 1767: 930, 943. Gmelin 1789: 2723, redescription. Christ 1791: 301, diagnosis. catalog.

Evania appendigaster de Geer 1773: 594-596.

Ichneumon niger de Geer, 1773: 594. Synonymized by Olivier 1792: 453.

Evania laevigata Olivier, 1792: 452. Synonymized by Schletterer 1886: 12.

Evania unicolor Say, 1824: 57-58. Synonymized by Dalla Torre 1902: 1077.

Evania desjardinsi Blanchard, 1840: 299. Male, Mauritius, (? MNHN). Synonymized by Schletterer 1886: 12.

Evania fuscipes: Spinola 1840: 246 . Synonymized by Dalla Torre 1902: 1077.

Evania affinis Le Guillou, 1841: 326. Hawaii, Maui, Hamoa (? MNHN). Synonymized by Schletterer 1886: 12.

Evania desjardinsii {sic}: Westwood 1843: 242. Synonymized by Dalla Torre 1902: 1077-1078.

Evania cubae Guérin-Méneville, 1844: 405. Cuba, type(s) lost?. Synonymized by Cresson 1865: 8.

Evania peringueyi Cameron, 1906: 19-20. ♂, ♀, Cape Town, Cape colony (NHM, Female, type # 3.a.291). Synonymized by Brues 1924: 8.

Evania abrahami Joseph, 1952: 52. ♀. Holotype ♀: India, Uttar Pradesh, Agra (IARI). **Syn. nov.** (Type examined) (Fig. 2)

Evania agraensis Joseph, 1952: 47-48. ♀. Holotype ♀: India, Uttar Pradesh, Agra (IARI). **Syn. nov.** (Type examined) (Fig. 3)

Evania johni Joseph, 1952: 48-49. ♀. Holotype ♀: India, Uttar Pradesh, Agra (IARI). **Syn. nov**. (Type examined) (Fig. 4)

Evania nurseana Cameron, 1906: 99-100. ♀, ♂. Syntype male, female: Pakistan, Quetta (? BMNH). **Syn. nov.** (Type photographs not examined)

Evania rubrofasciata Brues, 1916: 718-719. ♂, ♀. Type not designated Male, Female: India, Tamil Nadu, Vellore (=North Arcot district) (? Type lost). **Syn. nov.**

Evania simlaensis Cameron, 1909: 660-661. ♀. Holotype ♀: India, Himachal Pradesh, Shimla(=Simla) (? BMNH). **Syn. nov.** (Type photographs examined www.evanioidea.info)

Evania trivandrensis Joseph, 1952: 45-46. ♂. Holotype ♂: India, Kerala, Trivandrum (IARI). **Syn. nov.** (Type examined) (Fig. 5)

Evania unipunctata Joseph, 1952: 50-51. ♀. Holotype ♀: India, Uttar Pradesh, Agra (IARI). **Syn. nov.** (Type examined) (Fig. 6)

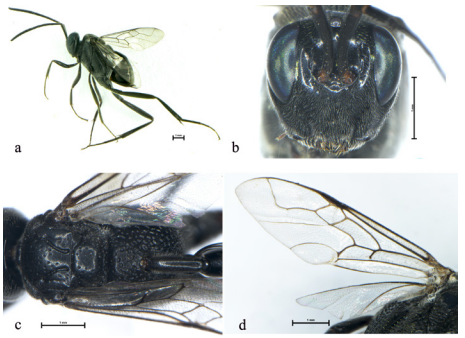


Fig 1. *Evania appendigaster* (L.). a) Habitus, lateral; b) Head, frontal view; c) Mesosoma, dorsal view; d) Fore wings.

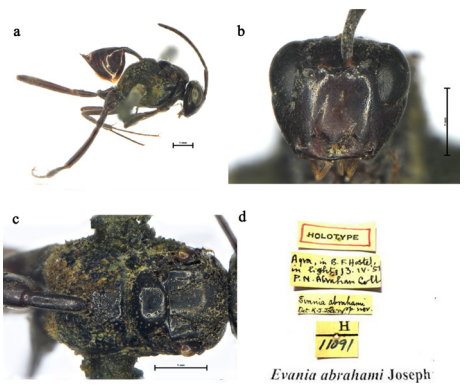


Fig 2. *Evania abrahami* Joseph. a) Lateral habitus; b) Head, front view; c) Mesosoma, dorsal view; d) Original label.

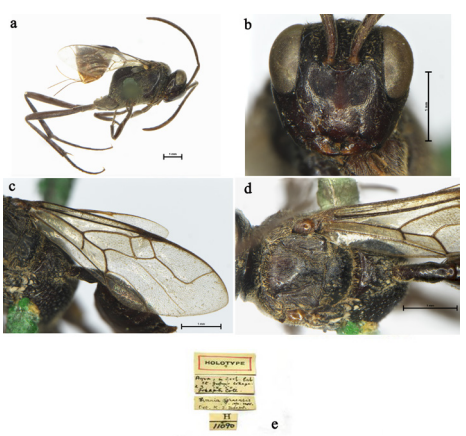


Fig 3. *Evania agraensis* Joseph. a) Lateral habitus; b) Head, front view; c) Fore wing; d) Mesosoma, dorsal view; e) Original label.

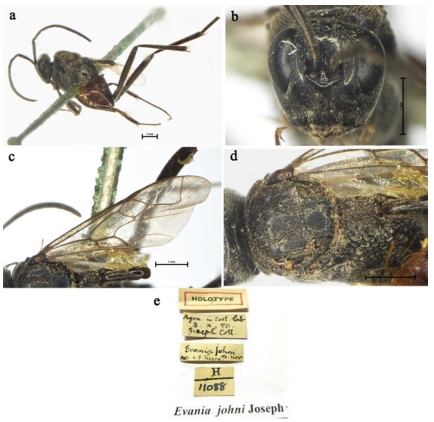


Fig 4. *Evania johni* Joseph. a) Lateral habitus; b) Head, front view; c) & d) Mesosoma, dorsal; e) Original label.

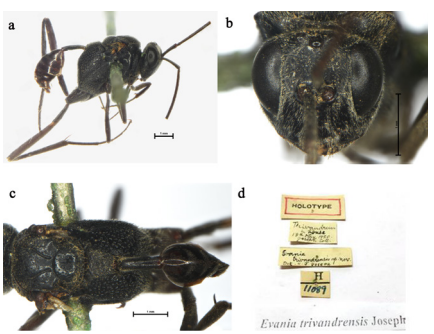


Fig 5. *Evania trivandrensis* Joseph. a) Lateral habitus; b) Head, front view; c) Mesosoma and metasoma, dorsal view; d) Original Label.

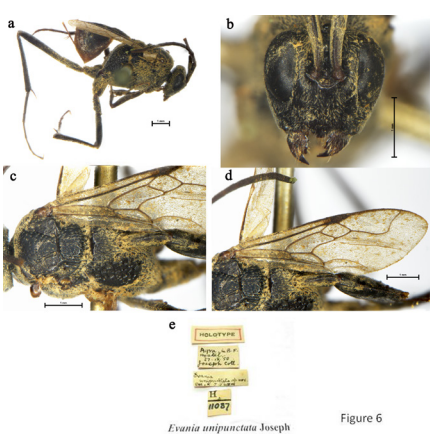


Fig 6. *Evania unipunctata* Joseph. a) Lateral habitus; b) Head, front view; c) Mesosoma, dorsal view; d) Fore wing; e) Original label.

Diagnosis. Female. Body length 7.1-7.9 mm. Body dark brown to black; head, frontovertex and antenna dark brown with silvery eyes; ocellus nitid brown; antennal socket nitid brown; tegula nitid brown; wing hyaline, venation nitid dark brown; all legs dark brown to black; ¼ basal of metasoma, exserted part of ovipositor nitid brown.

Head. Head high as wide; frontovertex smooth with silvery setae, 0.6 × head width and 1.7× scape length; mandibles bidentate; malar space about 0.4× eye length; OOL 0.5× POL; ocelli enlarged; face without distinct shallow longitudinal depression on each side of median line; clypeus convex with silvery setae; antennal scape 5.8× as long as broad; F1 longer than F2; pedicel 1.5× as long as broad; median ridge between antennal insertion, reaching almost to anterior ocellus; antennal socket almost middle of head. Relative measurements: head width (length), 2.3(2.3); frontovertex width, 1.4; distance between two posterior ocelli, 0.2; distance between posterior ocellus and eye margin, 0.4; eye length (width), 1.3(0.75); malar space length, 0.5; scape length(width), 1.7(0.3); pedicel length (width), 0.3(0.2).

Mesosoma. Weakly punctuate; mesoscutum 0.6× longer than wide; parapsidal furrow widening posteriorly, not touching posterior margin; scutellum sparsely areolate, 1.5× wider than long; propodeum foveae; legs covered with minute setae; hind tibia slightly shorter than hind femora, almost equal to combined length of hind tarsomere(1-5); internal spur of hind tibia almost equal to or slightly shorter than external spur; tarsal claws each with two teeth; length of 5th tarsomere 1.6× length of claws length. Relative measurements: mesosoma length, 3.9; mesoscutum length (width), 1.3(2.0); scutellum length (width), 0.8(1.2); hind femur length, 4.0; hind tibia length 3.8; hind tarsal length, 3.9; hind tibia internal spur, 0.6; hind tibia external spur, 0.5; 5th tarsomere length, 0.5; tarsal claws length, 0.3.

Wings. Hyaline; setose, but more so apically; veins C, Sc+R, 1M, RS+M, 2RS, M+CU, 1CU-a, 1A, 2A, 2CU, 2Mb, 3M, 2CU-a and 3A present; fore wing and hind wing both 2.9× longer than wide; 13 hamuli; jugal lobe present. Relative measurements: fore wing length (width), 6.5(2.2); hind wing length (width), 4.1(1.4).

Metasoma. Triangular, 1.1× longer than wide; petiole about half of metasoma, carinate, 3.7× longer than wide and 0.4× length of metasoma; ovipositor slightly exserted. Relative measurements: Metasoma length (width), 3.1(2.6); petiole length (width), 1.5(0.4).

Male. Body finely punctate except propodeum and metasoma; head 1.07 × wider than long; frons and facial area surrounding antennal sockets densely setose with short white hairs and sparsely punctate with shallow punctures; ocelli dark brown, nearly equal in size, OOL 1.7 × as long as POL; clypeus convex; densely setose; posterior margin of clypeus not defined; head width 1.6 × width of frontovertex; gena flat, densely setose; mandibles bidentate; median ridge between antennal insertion, reaching almost to anterior ocellus; pre-orbital antennal carina running with median groove; antennal groove depressed and with two lateral depressions on either side of antennal groove and eyes; antennal sockets within upper third of the head; scape pedicel and flagellomeres black; scape covered with minute setae with white hairs, 3.2 × length of pedicel.

Mesosoma. Black, weakly punctate anteriorly, broadly foveolate posteriorly; pronotum setose, and with sparse, large punctures; propleuron with anterior and ventral portions smooth, but transversely rugose posteriorly; mesoscutum 1.2× wider than long, densely setose with white hairs, and with scattered, wide punctures; parapsidal furrow widening posteriorly, not extending to posterior margin of mesoscutum; scutellum 2.0× wider than long, sparsely aerolate, and densely setose with white hairs; metanotum with single row of concentric foveae; mesopleuron swollen except for rugose part posteriorly; metapleuron densely foveolate, setose; propodeum shiny, with scattered foveae dorsally and sides concentrically strigate-areolate. Legs black, covered with setae; hind tibia slightly longer than hind femur; hind tibia 1.03× combined length of tarsomeres 1–5; tibial spur black, length of interior spur 1.6× length of exterior spur; each tarsomere with stiff spines apically; tarsal claws each with two teeth, 14.6× length of tarsomere 5.

Wings. Hyaline; setose, but more setose apically; veins black, C, Sc+R, 1M, RS+M, 2RS, M+CU, 1CU-a, 1A, 2A, 2CU, 2Mb, 3M, 2CU-a and 3A present; 10 hamuli (9 apically and 1 basally); jugal lobe present.

Metasoma. Elliptical, setose dorsally; petiole 4.2× longer than wide, arching dorsally and setose; metasomal tergites II-IX, ovoid, nitid.

Hosts. Cockroach ootheca of the subfamily Blattinae (Blattidae) (Westwood, 1843; Roth, 1989; Westwood, 1843; Lit, 1988, Yeh & Mu, 1994).

Distribution. Widely distributed throughout Afrotropic, Indomalayan, Palearctic (Greece, Turkey and north of Sweden except eastern Russia), Australasia, Nearctic and Neotropical except Antarctica.

Material examined: INDIA: UTTARAKHAND, Chamoli dist., Joshimara, 1♀, 16.09.1979, Coll. S.K. Gupta & Party; ANDHRA PRADESH, Sadwal, 1♂, 14.12.1987, Coll. B. Nauri & Party; Ananthapur dist., Amegundapalayam, 1♀, 20.08.2014, Coll. S. Prabakaran & Party. KERALA, Kozhikode dist., ZSI Campus, 1♀, 16.02.2005, 1♀, 27.09.2010, Coll. Madhavan; 4♀♀, 1♂, 30.vi.2005, Coll. K.C. Gopi; Kozhikode dist., East Hill, 1♀, 28.viii.2006, Coll. K.C. Gopi; Kozhikode dist., Malapparamba, 1♀, 23.02.2010, Coll. Mercy; Thiruvannur, 4♀♀, 1.09.2005, Coll. K. Rajmohona; Malappuram dist., Nilambur, 1♀, 10.02.2006, Coll. K. Rajmohona; Malappuram dist., Mampad, 1♂, 1♀, 31.xii.2007, Coll. S. Santosh; Calicut, 1♂, 15.12.2006, 1M, 9.05.2008, 1♀, 15.09.2008, Coll. K.C. Gopi; Kozhikode dist., Nanminda, 1♂, 12.01.2007, 1♀, 5.10.2011Coll. Girish Kumar, P.; Calicut, 1♀, 9.05.2008, Coll. K.C. Gopi; Calicut University Campus, Malappuram dist., 1♂, 28.12.2007; Kadalundi, 1♂, 28.11.2008, Coll. K.G. Emmeliamma; Trivandram, 1♀, 18.05.1950, Coll. Joseph; WEST BENGAL, Kolkata, Lake Gardens, 1♀, 12.05.2007, Coll. Girish Kumar, P; Kolkata, 1♀, 25.06.2008, 1♂, 27.06.2008, 1♀, 9.07.2008, 1♂, 9.08.2008, Coll. Girish Kumar, P.; Kolkata dist., Southern Avenue, 1♂, 16.04.2009, 1M, 26.04.2009, 1♀, 9.09.2009, Coll. Girish Kumar, P.; Kolkata dist., New Alipore, ZSI Campus, 1♂, 15.06.2018, 1♀, 18.06.2018, Coll. Paromita Mandal; 1♀, 3.08.2018, 1♂, 16.07.2018, 1♀, 25.08.2018, 1♂, 31.07.2018, Coll. Shankhamala Ghosh; 1♀, 9.07.2018, Coll. Chandan Bera; 1♂, 13.06. 2019, Coll. S. I. Kazmi; UTTAR PRADESH, Agra,B. F. Hostel, 1♀, 13. 04. 1951, Coll. P. N. Abraham;1♀, 27.09.1950, Coll. Joseph; Agra, St. John's College, Zoology lab.,1♀, 23.09.1950, 1♀,3.10.1950, Coll. Joseph.

Remarks. After examining the type materials, photographs (except *E. nurseana*) (www.evanioidea.info) and original descriptions of *E. abrahami*, *E. agraensis*, *E. johni*, *E. nurseana*, *E. rubrofasciata, E. simlaensis*, *E. trivandrensis*, *E. unipunctata,* it is found that all the above-mentioned species are synonyms of *Evania appendigaster*. Deans (2005) also indicated this synonymy.

***Evania mukerjii* Mani** (Fig. 7)

Evania mukerjii Mani, 1943:13-14. ♂. Holotype ♂, India: Delhi (IARI), (Type examined).

Redescription. Male. Body length 8.0 mm.

Head. Dark brown and smooth; ocelli and eyes black; genae smooth; face with silvery hair; antennal segments dark brown. Head wider than long; frontovertex smooth, about half of head width, 1.8× scape length; POL 2× OOL and AOL posterior ocelli bulges; malar space slightly longer than POL, equal to scape length; eyes 2.1× longer than wide; mandible 2-dentate; face convex with a median longitudinal ridge connecting anterior margin of clypeus; antennal sockets present upper third of head; antennal segments longer than broad; F1 slightly longer than scape and pedicel combined; scape 2.5× longer than broad; pedicel almost quadrate. Relative measurements (in mm) - Head width (height), 1.7(2.0); frontovertex width, 0. 9; POL, 0.4; AOL, 0.2; OOL, 0.2; eye length (width), 1.3 (0.6); malar space length, 0.5; scape length (width), 0.5 (0.2); pedicel length (width), 0.15(0. 2); F1 length, 0.7.

Mesosoma. Orange, weakly foveate on dorsal side; tegulae orange brown; parapsidal furrow present; mesoscutum 0.8× longer than broad; scutellum 0.58× longer than broad; scutellum and propodeum separated by a deep groove on dorsal side; mesopleuron with minute puncture. Legs: brown to dark brown; hind tibia longer than hind femur, 1.3× longer than mesosoma hight; spines present on dorsal surface of hind tibia; longer spur of hind tibia slightly less than half length of metatarsus. Relative measurements (in mm) - Mesosoma height, 2.2; mesoscutum length (width), 1,2 (1.5); scutellum length (width), 0.7(1.2); hind coxa length, 1.0; hind trochenter length, 1.1; hind femur length (width), 2.6 (0.4); hind tibia length (width), 3.0 (0.3).

Wings. Hyaline; 7 complete cells; RS+M, 3M, CU vein present; fore wing 3.0× longer than wide. Relative measurements: fore wing length (width), 6.0(2.0).

Metasoma. Dark brown, elliptical, smooth nitid, about 2.0×longer than broad; petiole smooth, anterior half or more red, posterior half dark brown, 0.52× broader than metasoma, 4.3× longer than broad. Relative measurements (in mm) - Petiole length (width), 1.3(0.3); metasoma length (width), 2.5(1.3).

Female. Unknown.

Hosts. Unknown.

Distribution. Indomalayan region (INDIA: Delhi).

Material examined: Holotype. INDIA: Delhi, IARI campus, 1 ♂, 4.viii. 1939, Coll. Mukerjii.

Remarks. *E. mukerjii* was recorded from India by Mani (1943) based on the male. Unfortunately, during the survey, we were unable to collect the specimen. However, after examining the holotype and original description by one of us (SIK), it clearly shows that this species is very distinct from male *E. appendigaster* mainly by body colour, the ratio between intraocular space and ocellocular space, petiole sculpture and ratio between a longer spur of hind tibia and metatarsus.

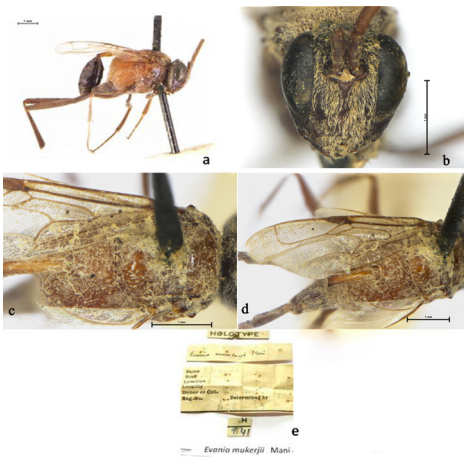


Fig 7. *Evania mukerjii* Mani. a) Lateral habitus; b) Head, front view; c) Mesosoma, dorsal view; d) Fore wing; e) Original label.

DISCUSSION

To date, two species of *Evania* have been recognized in India. Descriptions of both species are provided here. The cosmopolitan species of *Evania appendigaster* (L.) is misidentified as a new species by various authors in different periods due to variations. Many species described under the genus *Evania* from India are either synonymy or transferred to other genera. A few other genera are currently being analyzed further and will hopefully yield species identifications or new species descriptions.

Evania abrahami: The original description of this species was based upon a single female holotype, and the distinguishing characters were longer spur of the hind tibia about 2/5th (*versus* about 1/3rd) length of the metatarsus, hind wing with 11 (*versus* 13) hamuli, and malar space half (*versus* slightly less than half) the length of eye length. The same is the case of *E. agraensis* where it was described based on a single female with distinguishing characters as the long spur of the hind tibia about 2/5th the length of the metatarsus, the hind wing with ten hamuli, and the malar space more than half the eye length; *E. johni* differs from *E. appendigaster* in having a long spur of the hind tibia about 2/5th the length of the metatarsus, the hind wing with ten hamuli, and the malar space more than half the length of eye length. After examining the type specimens of *E. abrahami*, *E. johni*, the characters mentioned are not supportive of making it different from *E. appedigaster.*

Evania agraensis: This species was described from a single female holotype and is indistinguishable from *E. appendigaster*. The differences between these two species are very few and fall within the range of variation of the species. The holotype of *E. agraensis* differs from other examined specimens of *E. appendigaster* in having the long spur of the hind tibia about 2/5th the length of the metatarsus, the hind wing with ten hamuli, and the malar space more than half the eye length.

Evania johni: The original description of this species was based upon a single female holotype, which is indistinguishable from *E. appendigaster*. The differences between these two species are very few and fall within the range of variation of this species. The holotype of *E. johni* differs from other examined specimens of *E. appendigaster* in having a long spur of the hind tibia about 2/5th the length of the metatarsus, the hind wing with ten hamuli, and the malar space more than half the length of eye length, as for the holotype of *E. agraensis*.

Evania nurseana: The species was described from both female and male specimens. *Evania nurseana is* very similar to other examined specimens of *E. appendigaster*, differing in having the interocular space about twice the ocellar space (*versus* one and a half times the ocellar space), malar space half the eye length (*versus* slightly less than half eye length), and longer spur of the hind tibia about half-length (*versus* about 1/3rd length) of the metatarsus.

Evania rubrofasciata: The original description of *E. rubrofasciata* was based on a single female and male and left no doubt that *E. rubrofasciata* and *E. appendigaster* are the same species. Given the wide distribution of species, it is not surprising that the species shows some variation in antennomeres' dimensions and the relative length of the hind tibial spur and metatarsus. In *E. rubrofasciata* the malar space is 1/3rd (*versus* slightly less than half) eye length, and the long spur of the hind tibia is 1/3rd (*versus* about 1/3rd) the length of the metatarsus.

Evania simlaensis: This species was incompletely described by Cameron based on a single female. The description of *E. simlaensis* is very close to that of *E. appendigaster*. However, it differs from *E. appendigaster* in having the malar space half the eye length (*versus* malar space slightly less than half) and the long spur of the hind tibia less than 1/3rd (*versus* about 1/3rd) length of the metatarsus.

Evania trivandrensis: The original description of this species was based on a single male and is virtually indistinguishable from *E. appendigaster*. The differences between the two species are minor and fall within the range of variation of this species. The holotype of *E. trivandrensis* differs from other examined males of *E. appendigaster* in having the interocular space about twice (*versus* 1.5 times) the ocellar space, the hind wing with 10 (*versus* 13) hamuli, and the malar space half (*versus* slightly less than half) the length of an eye.

Evania unipunctata: The original description of this species was based upon a single female and is indistinguishable from *E. appendigaster*. The holotype of *E. unipunctata* differs from other examined females of *E. appendigaster* in having interocular space about twice (versus 1.5 times) the ocellar space, the hind wing with 12 (versus 13) hamuli, and the malar space 1/3rd (versus slightly less than half) the eye length.

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Effects of Mineral Oils, Palizin and Buprofezin on Functional and Numerical Responses of Predatory Ladybeetle *Cryptolaemus montrouzieri* (Mulsant, 1850)

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ABSTRACT

Predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant is an important biological agent in citrus orchards. The effect of six treatments included: two types of mineral oils, EC and mayonnaise at the concentration of 15 ml/L, buprofezin 0.75 ml/L, buprofezin+EC oil 0.5+5 ml/L, insecticidal soap palizin 2.5 ml/L, and water as the control were investigated on the functional and numerical responses of *C. montrouzieri* Mulsant under laboratory conditions. The experiment investigated predation rate of female adult coccinellids to varying densities (2, 4, 8, 16, 32 and 64) of mealybug pray, *Pseudococcus citri* Risso. The relationship between the rate of consumption and pray density was explored with a logistic regression. The results of logistic regressions revealed that the rate of prey consumption by the predator had risen as prey density increased, but eventually leveled off in both the control and all insecticide treatments (following Holling's type II functional response). Comparison of attack rates showed that the parameter significantly decreased when the predator was exposed to all insecticides but palizin. Predator handling times for oil mayonnaise, buprofezin and buprofezin+ EC oil treatments were significantly longer than that of the control. The longest handling time (1.6065 h) and the lowest attack rate (0.0351 h^{-1}) were estimated for buprofezin+oil and oil EC treatments, respectively. As prey density increased, increase in oviposition and decrease in the food exploitation efficiency were observed. The results confirmed that the functional response of *C. montrouzieri* was affected by tested compounds reducing the predatory potential. The exception was palizin that had a low effect on functional response events of treated *C. montrouzieri*.

Key words: predatory coccinellid, mealybug, insecticides, functional response, numerical response.

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INTRODUCTION

Cryptolaemus montrouzieri Mulsant (Coleoptera: Coccinellidae) is an important biological control agent of citrus scales *Planococcus citri* Risso and *Pulvinaria aurantii* Cockerell in citrus orchards around the world, including Iran (Heidari & Copland 1992; Aghajanzadeh, Halagisani, & Gholamian, 2016). Both larvae and adults of this ladybird feed on the mentioned pests affecting their population growth (Saljoqi et al, 2015).

C. montrouzieri is very sensitive to many chemical compounds used in citrus orchards for pest control. The coccinellid can be affected by insecticide sprays which decrease its predation efficiency (Aghabaglou, Alvandy, Goldasteh, & Rafiei, 2013). Many insecticides such as chlorpyrifos, diazinon, buprofezin, malathion, gozathion and etrimfos (Nourbakhsh, 2020), mineral oils (Rajabpour, Seraj, Damavandian, & Shishebor, 2008) and insecticidal soaps (Halagisani, Naseri, Rafiee Dastjerdi, Aghajanzadeh, & Ghadamyari, 2019) are recommended against scale bugs in citrus orchards.

The functional response of a predator is one of the crucial factors in population dynamics of a prey-predator system (Saljoqi et al, 2015). Functional response tests show the potential of a predator's ability to suppress different prey densities. In fact, the functional response relates to the change in predation rate with changing prey density and describes the way natural enemies responds to the changing densities of its prey (Holling, 1959). Predator's attack rate (the rate of successful attack of prey by a predator; also knows as searching efficiency or the rate at which a predator searches for finding its prey) and handling time (as a time it takes for a predator to encounter and consume a single prey) are two important parameters of functional response to evaluate the effectiveness of predators (Omkar & Kumar, 2013). In addition to the functional response, numerical response is another component of the predator-prey interactions and is defined as the change in predator numbers with the variation in prey density (Solomon, 1949). Progeny production by predator females can change in relation to prey density (Rahmani Piyani, Shishebor, Kocheili, & Eric, 2021). The functional response is known to be the key factor and defines the numerical response (Keith, Todd, Brand, Adamcik, & Rusch, 1977). The intake rate of a predator is described as a function of food density. Depending on the assumptions Holling (1959) separated functional response into three differently modelled types (type I, II and III). Most natural enemies show type II and III of functional response (Holling, 1959). Type I corresponds to the consumption of prey by the predator resulting in a linear increase. Type II occurs when the rate of predation decelerated reaching an asymptote. Type III involves a sigmoid relation also called S ascension (Santos et al, 2021).

The analysis of both the functional and numerical responses is helpful and provides information regarding the prey-predator relationship, which is required for the successful usage of a biological control agent (Rahmani Piyani, Shishebor, Kocheili, & Eric, 2021).

Several studies showed that insecticides adversely affect the functional and numerical responses of natural enemies. He, Zhao, Zheng, Desneux, & Wu (2012) reported that lady beetle, *Serangium japonicum* Chapin exposed to imidacloprid showed prolonged handling time and decreased attack rate while feeding on eggs of

Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae). Two insecticides, imidacloprid and chlorpyrifos decreased attack rate and increased handling time of the predator *Macrolophus pygmaeus* (Hem.: Miridae) that had been feeding on *Tuta absoluta* (Lep.: Gelechiidae) (Sharifian, Sabahi, & Bandani, 2017). The numerical response of *Amblyseius largoensis* Muma (Acari: Phytoseiidae) was affected by exposure to abamectin, where just 60% of the females oviposited (Barros et al, 2022). Many studies had been done on the predatory behavior of *C. montrouzieri* (Jamila, 2011; Abdolahi Ahi et al, 2012; Saljoqi et al, 2015; Qin, Wu, Qiu, Ali, & Cuthbertson, 2019), but there are only a few information on the effect of pesticides on the type and parameters of the functional response (Pakyari, Kasirloo, & Arbab, 2016). Torres & Marcano (2015) tested the numerical response of *C. montrouzieri* to seven densities of third instar nymphs of *Maconellicoccus hirsutus* Green. However, no research has been done on the effect of pesticides on the numerical response of *C. montrouzieri.* In this study, the effects of two types of mineral oils, mayonnaise and emulsifiable concentrates (EC), buprofezin, buprofezin + oil combination, and the insecticidal soap palizin (the widely used insecticides in citrus orchards) examined on the functional and numerical response of *C. montrouzieri* to *P. citri*. The purpose of the study was to determine the type of functional response and estimate the parameters and the reproductive response of females of *C. montrouzieri* when affected by the pesticides. It is important in evaluating the performance of *C. montrouzieri* to a changing prey density and forecasting the suitability of the predator as a potential biocontrol agent. The information obtained would be useful in integration of the pesticides with the predatory coccinellid in integrated pest management of *P. citri*.

MATERIALS AND METHODS

Insect rearing

The colonies of the mealybug, *P. citri* and the coccinellid, *C. montrouzieri* were obtained from the insectarium in Amol, Mazandaran province (North Iran) in May 2018. The mealybug was reared on pumpkin, *Cucurbita moschata* Duchesne ex Poir. (Cucurbitacae) at the temperature of 26±1 ºC, with 60±10% R.H and 16:8 L: D photoperiod in a growth chamber. Every two weeks, new fresh pumpkins were added to the mealybug colony for the continuous supply of *P. citri.* The larvae of *C. montrouzieri* were kept in plastic containers (3×7 cm^2) in a rearing chamber and were provided with a daily diet of *P. citri*. After emergence, adults of *C. montrouzieri* were transferred to Petri dishes (60 mm in diameters). Egg sacs of mealybug were used as an oviposition substrate. After the copulation and egg laying, the eggs of *C. montrouzieri* were collected with a hair brush and cultured until the emergence of new adults. The same age adults were used for the functional response experiment.

Chemicals

The compounds used in this study are mineral oils - EC (Ghazal Shimi, Iran, degree of sulphonation: 92% (90% chlorinated paraffin mineral oil + 10% emulsifier)

and mayonnaise (Cayan, Iran, degree of sulphonation: 80% (80% chlorinated paraffin mineral oil + 18% water+ 2% emulsifier), buprofezin insecticide (Nihon Nohyaku, Japan, 40% SC), and insecticidal soap, palizin (Kimia sabzavar, Iran, Coconut soap 65%).

Bioassay

The insecticides were used at recommended field concentrations (Nourbakhsh, 2020). Mineral oils: EC and mayonnaise at the concentration of 15 ml/L, buprofezin 0.75 ml/L, buprofezin+oil (EC) 0.5+5 ml/L, and insecticidal soap palizin 2.5 ml/L. For bioassay, two hundred (1 day old) female and male adults of *C. montrouzieri* were used in each treatment. They were separated in different Petri dishes (60 mm in diameters) and placed into groups of 10 individuals per dish. Before the treatment, Petri dishes were placed in a refrigerator for 20 min to reduce the mobility of ladybeetles. Then each Petri dish containing adults of *C. montrouzieri* was sprayed with 1 ml of insecticide solution according to Karamaouna et al, (2013). Control Petri dishes were sprayed with water. After spraying, the excess solution was removed and Petri dishes were kept under laboratory conditions at 25±1°C, 65% RH and a 16:8 light: dark photoperiod.

Functional response experiment

Twenty-four hours after the treatment, only the surviving females of *C. montrouzieri* were collected for use in the functional response experiment. The surviving males were transferred to other Petri dishes with mealybugs as food and kept until numerical response experiment. With a mortality ≤ 40 percent for insecticide treatments in the bioassay, three hundred sixty females were used in the functional response experiment (sixty female adults in each treatment). Adults were starved for another 12 hours and then individually transferred to Petri dishes (60 mm in diameters). Mealybugs of the same age were used as prey for ladybeetles at different densities (2, 4, 8, 16, 32 and 64). After 24 h, the predators were removed and the number of consumed prey (Na) was recorded. Each density was replicated 10 times (10 female adults in each prey density) for insecticide treatments and the control. All experiments were carried out under laboratory conditions as mentioned above.

Numerical response

For the numerical response experiment, three hundred sixty females from the functional response part were used (sixty female adults in each treatment). Females from each prey density were mated with the surviving treated males of the same age. Within 24 hours, after observing mating activity, females were transferred to separate Petri dishes provided with different densities of prey (2, 4, 8, 16, 32 and 64). Petri dishes were monitored daily and the number of eggs laid by females was counted. After removing the eggs for daily counting and prevention of cannibalism, each female was transferred to a new Petri dish containing the same prey density as before. With an average of 7.58 days pre- oviposition period, the number of eggs laid by each female was recorded daily for 15 days (Therefore, the whole experimental procedure including the functional response, time for the mating and the numerical response, lasted 17 days). The experiment was replicated ten times (10 female adults in each

prey density). Also, food exploitation efficiency in females was calculated using the following equation (Omkar & Kumar, 2013):

Food exploitation efficiency (%)= number of prey consumed/number of prey offered×100

Data analysis

The type of the functional response was determined by logistic regression analysis (SAS/STAT, CATMOD procedure version 9.0) of the proportion of prey killed (Na) in relation to the initial prey density (N0) (Trexler & Travis 1993). The data were fitted to the logistic regression which describes the relationship between Na/N0 and N0 (Juliano, 2001):

$$\frac{N_a}{N_0} = \frac{\exp\left(P_0 + P_1 N_0 + P_2 {N_0}^2 + P_3 {N_0}^3\right)}{1 + \exp\left(P_0 + P_1 N_0 + P_2 {N_0}^2 + P_3 {N_0}^3\right)}$$

Where P_0, P_1, P_2, and P_3 are the intercepts of linear, quadratic and cubic coefficients, respectively, and estimated using the method of maximum likelihood. If the linear parameter P_1 is negative, a type II functional response is evident, whereas a positive linear parameter indicates density-dependent predation and thus a type III functional response (Juliano, 2001). After the determination of the shape of the curve, the handling times and attack coefficients of a type II response were estimated using Holling's disk equation (Williams & Juliano, 1985):

$$N_a = N_0(1-\exp(a(T_h N_a - T)))$$

Where N_a is the number of preys attacked, a is the attack rate, N_0 is initial prey density, T is the total available time and T_h is the handling time.

Pairwise comparisons of functional responses parameters were performed using the indicator variable method (Juliano, 2001):

$$N_a = N_0\{1 - \exp[\frac{-a + Da\,(j)(T)}{(1 + (a + Da(j))(Th + DTh(j))(N_0)}]\}$$

Where j is an indicator variable that takes on a value of 0 for the first data set and one for the second data set. The parameters, D_a is a difference in the attack rate and D_{Th} is a difference in the handling time between two treatments. In other words, the attack rate for one treatment is a, and that for another treatment is D_a. Testing for a significant difference in attack rates between two treatments is accomplished by testing the null hypothesis that D_a=0 (Juliano, 2001, Allahyari, Fard, & Nozari, 2004). To find the difference between two handling times (for control and treatment), it must be proved that D_{Th} is not equal to zero. If D_{Th} isn't significantly different from zero, the difference between T_h and T_h+D_{Th} is not significant and the two handling times are not statistically different (Juliano, 2001).

RESULTS

Consumption-prey density

The relationship between the number of prey density and the number of prey consumed for all treatments is showed in Fig. 1. In this figure, with the increase in the prey density**,** functional response decreases exponentially. The number of killed mealybugs was significantly higher in the control treatment than in insecticide treatments at all densities. The mean number of mealybugs that are consumed by *C*. *montrouzieri* at the highest prey density (64 mealybugs) was 21.5, 15, 16.2, 13.2, 11.2, and 20 mealybugs/predator in the control, EC oil, mayonnaise oil, buprofezin, buprofezin+ oil, and palizin treatments, respectively. There were significant differences in the number of mealybugs killed by treated *C*. *montrouzieri* at different prey densities (4 mealybugs: F= 6.76, df = 5,54, p< 0.0001; 8 mealybugs: F = 24.27, df = 5,54, p< 0.0001; 16 mealybugs: F =16.43, df=5,54, p< 0.0001; 32 mealybugs: F =28.99, df = 5,54, p< 0.0001, and 64 mealybugs: F =26.54, df = 5,54, p < 0.0001).

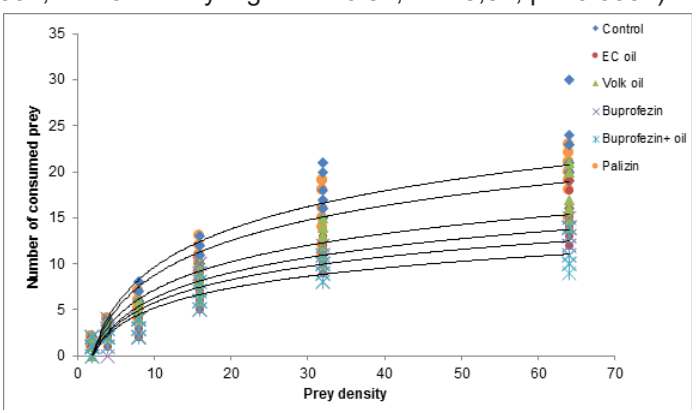

Fig. 1. Functional response of predatory coccinellid, *Cryptolaemus montrouzieri* exposed to the insecticides to different densities of mealybug, *Pseudococcus citri.*

Attack rate/handling time-treatment

Estimated parameters of the logistic regression for the functional response for all treatments are presented in Table 1. Since the linear parameter (P_1) was negative, the functional response for all treatments was type II. The values of attack rate (a) and handling time (T_h), estimated by Rogers' random attack equation are presented in Table 2. Insecticides caused adverse effects on functional response parameters of treated *C. montrouzieri* compared to control ladybeetles. The greatest attack rate value (0.0919 h^{-1}) and the shortest handling time value (0.9006 h) were obtained in the control treatment (Table 2). The longest handling time value (1.6065 h) and the lowest attack rate value (0.0351 h^{-1}) were estimated for buprofezin+oil and oil EC treatments, respectively (Table 2). Differences of functional response parameters of treated *C. montrouzieri* are presented in Table 3. Comparison of attack rates showed that the parameter significantly decreased when predator exposed to all insecticides

but palizin (Table 3). Handling times for oil mayonnaise, buprofezin and buprofezin+oil treatments were significantly longer than that for the control. However, values for the attack rate and the handling time of *C. montrouzieri* treated by palizin were lower and longer than those for the control, but without significant statistical differences. Results of comparing functional response parameters of the two types of oils showed a significant difference for attack rates but no statistically *significant difference for* handling times. However, adding oil to buprofezin decreased attack rate and increased handling time to buprofezin treatment, but without a significant difference.

Table 1. Estimated parameters of the logistic regression for functional response of treated coccinellid, *Cryptolaemus montrouzieri* to different densities of mealybug, *Planococcus citri*.

Treatment	Parameters	Estimate	SE	χ^2	p
Control	Intercept	2.0183	0.2754	53.73	0<.0001
	Linear	-0.0736	0.0166	19.71	0<.0001
	Quadratic	0.000492	0.000202	5.92	0.0150
	Cubic	-0.00004	0.000026	1.80	0.1796
Oil (mayonnaise) 15 ml/L	Intercept	0.8182	0.2236	13.39	0.0003
	Linear	-0.0487	0.0145	11.25	0.0008
	Quadratic	0.000298	0.000184	2.63	0.1047
	Cubic	-0.00002	0.000022	0.72	0.3971
Oil Ec 15 ml/L	Intercept	0.3620	0.2181	2.75	0.0970
	Linear	-0.0402	0.0145	7.72	0.0055
	Quadratic	0.000251	0.000185	1.86	0.1731
	Cubic	-0.00002	0.000022	1.06	0.3029
Buprofezin 0.75 ml/L	Intercept	0.2188	0.2181	1.01	0.3159
	Linear	-0.0362	0.0145	6.21	0.0127
	Quadratic	0.000183	0.000186	0.97	0.3254
	Cubic	7.683E-6	0.000021	0.13	0.7196
Buprofezin+ Oil Ec 0.5+5 ml/L	Intercept	-0.00758	0.2194	0.00	0.9724
	Linear	-0.0286	0.0147	3.77	0.0521
	Quadratic	0.000070	0.000189	0.14	0.7131
	Cubic	-3.89E-7	0.000022	0.00	0.9857
Palizin 2.5 ml/L	Intercept	1.5513	0.2481	39.10	0<.0001
	Linear	-0.0657	0.0154	18.10	0<.0001
	Quadratic	0.000455	0.000191	5.66	0.0174
	Cubic	-3E-7	0.000023	0.00	0.9897

Food exploitation-treatment-prey density

Food exploitation efficiency was decreased with an increase in prey density in each treatment (Table 4). There were no significant differences in food exploitation efficiency among treatments at density of 2 mealybugs whereas at the densities of 4, 8, 16, 32 and 64 mealybugs significant differences were observed (Table 4). At the highest prey density (64 mealybugs), the lowest food exploitation efficiency was obtained in buprofezin+oil treatment and without a significant statistical difference in buprofezin treatment.

Table 2. Coefficient of attack rate (a) and handling time (Th) estimated by Rogers random attack equation in treated ladybeetle, *Cryptolaemus montrouzieri* to different densities of mealybug, *Planococcus citri.*

Treatments	Parameters	Estimate	SE	95% CI	
				Lower	Upper
Control	a´ (h^{-1})	0.0919	0.0116	0.0687	0.1151
	T_h (h)	0.9006	0.0468	0.8068	0.9943
Oil (mayonnaise) 15 ml/L	a´ (h^{-1})	0.0501	0.00494	0.0402	0.0599
	T_h (h)	1.1137	0.0621	0.9894	1.2380
Oil EC 15 ml/L	a´ (h^{-1})	0.0351	0.00308	0.0289	0.0413
	T_h (h)	1.0887	0.0709	0.9468	1.2306
Buprofezin 0.75 ml/L	a´ (h^{-1})	0.0365	0.00387	0.0288	0.0443
	T_h (h)	1.3357	0.0885	1.1585	1.5128
Buprofezin+oil 0.5+5 ml/L	a´ (h^{-1})	0.0355	0.00384	0.0278	0.0432
	T_h (h)	1.6065	0.0987	1.4090	1.8041
Palizin 2.5 ml/L	a´ (h^{-1})	0.0715	0.00817	0.0552	0.0879
	T_h (h)	0.9441	0.0523	0.8394	1.0488

Table 3. Differences of functional response parameters estimated by an equation with an indicator variable in treated ladybeetle, *Cryptolaemus montrouzieri* to different densities of mealybug, *Planococcus citri.*

Treatments	Parameters	Estimate	SE	95% CI	
				Lower	Upper
Control- Oil mayonnaise	D_a	-0.0962	0.0431	-0.1814	-0.0109
	D_{Th}	0.2085	0.0856	0.0390	0.3779
Control- Oil EC	D_a	-0.1174	0.0405	-0.1977	-0.0372
	D_{Th}	0.1903	0.0989	-0.00570	0.3862
Control -Buprofezin	D_a	-0.1153	0.0417	-0.1979	-0.0328
	D_{Th}	0.4261	0.1162	0.1960	0.6562
Control -Buprofezin+oil EC	D_a	-0.1164	0.0406	-0.1968	-0.0359
	D_{Th}	0.6910	0.1366	0.4205	0.9616
Control -Palizin	D_a	-0.0577	0.0502	-0.1571	0.0417
	D_{Th}	0.0399	0.0733	-0.1053	0.1850
Oil EC- Oil mayonnaise	D_a	0.0213	0.00906	0.00332	0.0392
	D_{Th}	0.0182	0.0941	-0.1682	0.2047
Buprofezin-Buprofezin+oil	D_a	-0.00102	0.00774	-0.0163	0.0143
	D_{Th}	0.2649	0.1338	-0.00011	0.5300

Da is a difference in the attack rate and DTh is a difference in the handling time between two treatments.

Number of eggs-treatment-prey density

The number of eggs laid by females from each treatment was positively correlated with prey density (Fig. 2). With increasing prey density from 2 to 64, the number of eggs produced by a female was: in the control ($F_{5,54} = 45.7$; $P < 0.0001$), mayonnaise oil ($F_{5,54} = 40.3$; $P < 0.0001$), EC oil ($F_{5,54} = 50.94$; $P < 0.0001$), Buprofezin ($F_{5,54} = 40.65$; $P < 0.0001$), Buprofezin+oil ($F_{5,54} = 47.67$; $P < 0.0001$), and palizin ($F_{5,54} = 46.83$; $P < 0.0001$).

Table 4. Food exploitation efficiency (%) of treated *Cryptolaemus montrouzieri* females (n=10) at different densities of mealybug, *Planococcus citri*.

Densities	Treatments						
	Control	Oil (mayonnaise) 15ml /l	Oil EC 15 ml/L	Buprofezin 0.75 ml/L	Buprofezin+oil 0.5+5 ml/L	Palizin 2.5 ml/L	
2	85.0±7.6 a	75±11.1 a	75.0±8.3a	60.0±10.0a	55.0±13.8a	85.0±7.6a	P=0.1849 F5,54=1.57
4	92.5±3.8 a	67.5±5.3 abc	55.0±5.0 bc	57.5±9.8 bc	50.0±5.2 c	77.5±5.8 ab	P<0.0001 F5,54=6.76
8	81.2±3.8 a	58.7±3.2 bc	46.2±4.1 cd	37.5±3.7 d	37.5±3.2 d	72.5±4.0 ab	P<0.0001 F5,54=24.2
16	68.7±2.0 a	51.2±2.9 b	43.7±2.4 b	46.8±3.8 b	41.2±2.6 b	65.6±2.8 a	P<0.0001 F5,54=16.4
32	55.3±2.1 a	40.0±1.5b	34.6±1.1 bc	31.2±1.1 c	29.6±1.0 c	47.8±3.1 a	P<0.0001 F5,54=28.9
64	33.5±1.8 a	25.3±1.2 b	23.4±1.0 b	20.6±0.6 bc	17.5±0.8 c	31.2±1.1 a	P<0.0001 F5,54=26.5

Means in the row followed by the same letters are not statistically different (Tukey, p ≤ 0.05)

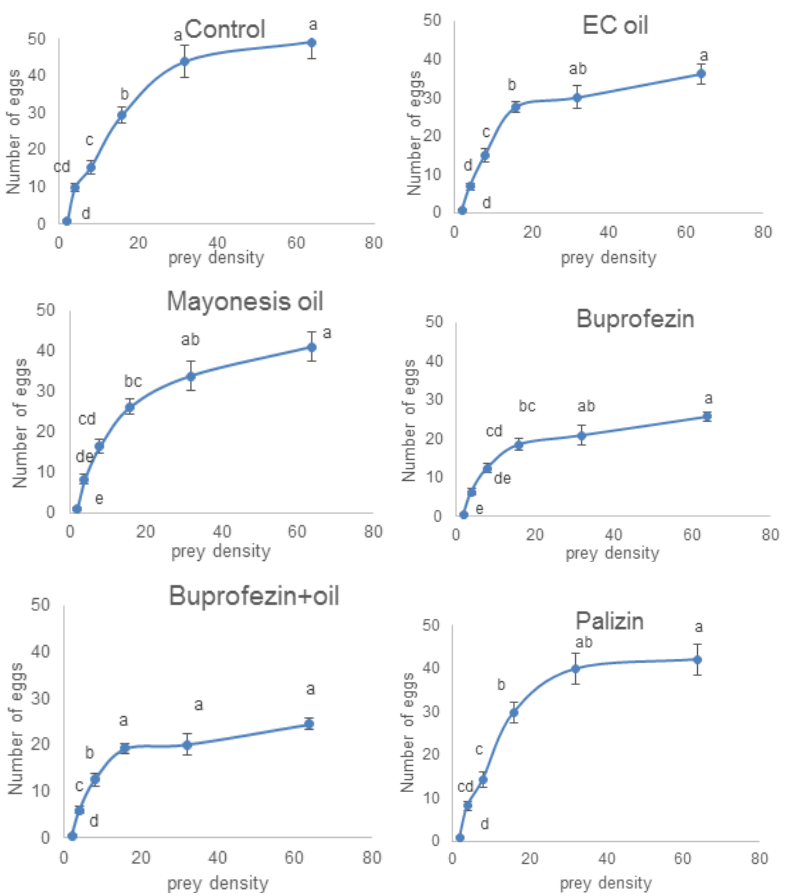

Fig. 2. The relationship between the number of eggs laid by females of *Cryptolaemus montrouzieri* and mealybug, *Planococcus citri* densities as prey

Points are mean ± SE. Means with different letters indicate significant difference (Tukey, p ≤ 0.05)

Table 5. Mean number (±SE) of eggs laid by treated *Cryptolaemus montrouzieri* females (n=10) at different densities of mealybug, *Planococcus citri*.

Densities	Treatments						
	Control	Oil (mayonnaise) 15 ml/L	Buprofezin 0.75 ml/L	Oil EC 15 ml/L	Palizin 2.5 ml/L	Buprofezin+oil 0.5+5 ml/L	
2	0.9±0.3a	1.0±0.3 a	0.8±0.3 a	0.5±0.3 a	0.4±0.2a	0.8±0.2a	P=0.73 F5,54=0.56
4	9.9±1.2a	8.3±1.3 a	6.8±1.0 a	6.3±0.8 a	6.0±0.7a	8.1±1.1a	P=0.12 F5,54=1.84
8	15.3±1.8a	16.4±1.8 a	15.1±17 a	12.4±1.3 a	12.5±1.4a	14.2±1.8a	P=0.49 F5,54=0.89
16	29.4±2.2a	26.2±1.9 ab	27.6±1.5 a	18.5±1.5 b	19.2±1.2b	29.7±2.4a	P<0.0001 F5,54=7.32
32	43.8±4.4a	33.9±3.6 ab	30.1±30 ab	20.9±2.5 b	20.1±2.2b	39.8±3.6a	P<0.0001 F5,54=8.48
64	49.0±4.2a	41.1±3.6 a	36.2±26 ab	25.7±1.3 b	24.5±12b	42.0±3.7a	P<0.0001 F5,54=9.92

Means in the row followed by the same letters are not statistically different (Tukey, $p \leq 0.05$)

Among treatments, there were no significant differences in the number of eggs laid at the densities of 2, 4 and 8 mealybugs (Table 5). Whereas at the densities of 16, 32 and 64 mealybugs, differences were statistically significant. At the density of 64 mealybugs, the lowest mean number of eggs laid by *C. montrouzieri* was related to buprofezin (25.7) and buprofezin+oil (24.5) treatments, and without a significant difference in the EC oil (36.2) treatment. The highest mean number of eggs was in the control treatment (49.0), followed by palizin treatment (42.0), while the differences in treatments with oils were not statistically significant.

CONCLUSIONS AND DISCUSSION

Prey consumption can be decreased when predators are exposed to different insecticides (Sharifian, Sabahi, & Bandani, 2017). Pesticides may interfere with the feeding behavior of exposed insects and cause the behavioral alterations in motility include lack of motor coordination, tremors, downfalls, abdomen tucking and rotational movement for abdomen cleaning (Suchail, Guez, & Belzunces, 2001). Leg and proboscis tremor of the predatory bug, *Andrallus spinidens* Fabricius (Hem.: Pentatomidae) after pesticides application was observed. This abnormal behavior increased the time that takes for a predator to encounter and consume a single prey and reduced prey consumption (GholamzadehChitgar, Hajizadeh, Ghadamyari, Karimi-Malati, & Hoda, 2014). In this study, treated *C. montrouzieri* with the insecticides captured fewer prey compared to the control. The more adverse effects were observed in the treatments that used buprofezin. Buprofezin might either inhibit the activity of some enzymes related to the activity of coccinellid or it might influence the neuroendocrine system, which might adversely affect predation (Gu, Bei, & Gao, 1993; Deng, Xu, Cao, & Dai, 2008). Prey consumption was less affected in palizin treated *C. montrouzieri* than in the oils treated the coccinellid. It may be because different chemical structures and modes of action of the two compounds which will be discussed in more detail in the following.

According to the negative values obtained for the linear parameters P_1, *C. montrouzieri* exhibited type II functional response on *P. citri* in all treatments. The proportion of prey consumed decreased as the density of prey increased may be due to satiation of the predator (Toft, 2005). Similar results for *C. montrouzieri* were reported by Ghorbanian (2010) and Abdollahi Ahi et al (2012). According to Pakyari et al (2016) functional response of *C. montrouzieri* adults on different densities of *P. citri* was type II at 27°C and 65±5% RH. Fourth instar larvae, female and male adults of *C. montrouzieri* showed type II when fed on different densities of cotton mealybug, *Phenacoccus solenopsis* Tinsley at 28±1°C and 65±5% RH (Saljoqi et al, 2015). Functional responses of adult males and females of *C. Montrouzieri*, and its 3rd instar larvae on *P. citri* were studied at seven constant temperatures ranging from 15 to 40°C, at % 60 ± 10 RH. Results showed that coccinellid adults and its 3rd instar larvae were type II, except for the 3rd instar larvae at 40°C, which were type III (Mohasesian, Ranjbar Aghdam, & Pakyari, 2015). Type II of functional response has also been demonstrated for coccinellids, *Oenopia conglobata* Linnaeus feeding on different densities of *Schizaphis graminum* Rondani (PahlavanYali & Bozorg-Amirkalaee, 2018) and *Harmonia axyridis* Pallas preying on crapemyrtle aphid, *Tinocallis kahawaluokalani* Kirkaldy (GholamzadehChitgar, 2019). Qin, Wu, Qiu, Ali, & Cuthbertson (2019) reported the functional response of *C. montrouzieri* predating on mealybug, *Dysmicoccus neobrevipes* Cockerell as type II. According to the results, insecticides exposure did not change the type of the functional response of *C. montrouzieri*. But the asymptote of the curve had a tendency towards lower values in insecticide treatments compared to the control. When treated with buprofezin+oil, asymptote of the functional response curve was lower than in treatments with other insecticides. A lower asymptote of treated coccinellids represents either a decrease in attack rate or an increase in handling time (Butt, Talib, & Khan, 2019). The predatory spider, *Oxyopes javanus* Thorell when exposed to sublethal concentrations of lambda cyhalothrin (95.5 a.i. µg/L), emamectin benzoate (125.14 a.i. µg/L) and imidacloprid (2475.1 a.i. µg/L) showed a lower asymptote in the functional response curves compared to the control, without a change in the type of a functional response in all treated and untreated groups (Butt, Talib, & Khan, 2019). Other studies also reported a significant decrease in prey consumption when natural enemies exposed to different insecticides without change in the type of functional response (GholamzadehChitgar, Hajizadeh, Ghadamyari, Karimi-Malati, & Hoda, 2014; Amini Jam, 2017; Sharifian, Sabahi, & Bandani, 2017). In contrast, exposure to imidacloprid affected the type of the functional response of the predator *Podisus nigrispinus* Dallas. *P. nigrispinus* presented a type III functional response in the absence of the insecticide, but the treatment with imidacloprid caused a type II response (Malaquias, Ramalho, Omoto, Godoy, & Silveria, 2014). *Coccinella septempunctata* L. larvae showed type II functional response after feeding on the *brassica aphids* (*Brevicoryne brassicae* L.) treated with thiamethoxam, lambda-cyhalothrin and cypermethrin, while imidacloprid, profenophos and chlorpyrifos treated aphids altered the functional response of larval coccinellids from type II to III. The change in response was linked to unconsciousness and disorientation induced by the insecticides targeting insect nervous system (Afza, Riaz, Afzal, & Majeed, 2021).

Feeding behavioral perturbations induced by insecticides with decreased capture, prolonged handling time and reduced attack rate negatively affect the efficiency of natural enemies (Ambrose, Rajan, & Raja, 2010). In this research, buprofezin+oil and oil EC treatments had the greatest effect on the functional response of *C. montrouzieri* compared to other insecticide treatments causing the longest handling time and the lowest attack rate, respectively. Oil in combination with buprofezin can have a synergistic effect and increase the diffusion and transport of insecticide molecules into the insect body (Mulrooney, Womac, & Greever, 1993). It can explain the more negative effect of buprofezin+ oil in comparison to buprofezin treatment on the predatory response of *C. montrouzieri*. Oils used in this study was petroleum products: stock emulsion type (mayonnaise) a polyphase product and miscible type (EC) a monophase product (Tajbakhsh, & Heidari, 2016). After application of oils on insect's body, they penetrate the cuticle, diffuse and accumulate within lipid-containing tissues, primarily the fat bodies. Accumulation in the nerve ganglia cause the direct effect of suppressing synaptic transmission in the insect's ganglia (Najar-Rodríguez, Lavidisd, Mensahb, Choyd, & Waltera, 2008). Behavior abnormalities such as receptor coating after oil spray may occur in treated insects and cause host location failures (Simons, 1982). The negative behavioral effects may result time-consuming activities that prolong handling time and reduce prey consumption. It can explain significantly longer handling time in oil mayonnaise treated *C. montrouzieri* compared to the control. But for oil EC treated *C. montrouzieri*, there was no statistically *significant difference in* handling times *with the control probably* depending on the type of oil. Many researchers have reported that insecticides exposure can affect the attack rate and the handling time of coccinellids. Pakyari et al (2016) showed that *C. montrouzieri* exposed to LC_{10} of fenpropathrin (7.63 µg a.i./ml) and LC_{30} of abamectin (2.10 µg a.i./ml) resulted in the highest handling time and the lowest attack rate, respectively. Imidacloprid residual (LC_{50}) increased handling time and decreased the attack rate of *S. japonicum* (He, Zhao, Zheng, Desneux, & Wu, 2012). On the other hand, palizin had a low effect on the functional response events of treated *C. montrouzieri* compared to the other insecticide treatments. Palizin as insecticidal soap (Potassium salts of fatty acids) dissolves the waxy layer on insect's body and damages the cell membrane. Saponification of the lipids in the cell tissues results in the cell membrane rupture and loss of vital fluids (Baniameri, 2008). Soft bodied insects such as aphids, whiteflies, and mealy bugs are more susceptible to desiccation. while, soaps have a minimal activity on some hard-bodied beneficial insects, such as adult lady beetles due to the insect's thickened cuticle (Weinzierl, 2000). A similar result associated with low harmful effects of palizin was reported by Amini Jam (2017) when the insecticidal soap effect was evaluated on the functional response of the parasitoid wasp, *Lysiphlebus fabarum* Marshall. The insecticide was effective against scale insects, *P. aurantii* and *P. citri* in citrus orchards (Ahmadi, Amiri-Besheli, & Hosieni, 2012; Halagisani et al, 2019) and relatively harmless to natural enemies (Kabiri & Amiri-Besheli, 2012; Gholamzadeh Chitgar, 2017).

In this study, with increased prey density, food exploitation efficiency decreased. This finding coincides with the research findings of Omkar & Kumar (2013) on food exploitation efficiency of females ladybeetle, *Anegleis cardoni* Weise in response to changing prey densities of *Aphis gossypii* Glover. Zarghami, Mossadegh, Kocheili, Allahyari, & Rasekh (2015) reported the reduction in food exploitation efficiency of *Nephus arcuatus* Kapur, with increasing density of its prey *Nipaecoccus viridis* Newstead. It was caused due to satiation of the predator after consuming a sufficient amount of the prey (Omkar & Kumar, 2013). Probably, a greater reduction on food exploitation efficiency of *C. montrouzieri* in the treatments that buprofezin was used, is due to its more adverse effect on altering predator behavior.

With increasing prey density, more eggs by per female in each treatment were produced. An increase in food quantity facilitates the development of more numbers of ovarioles affecting the egg production by the predator (Evans, 2000). Dixon & Guo, (1993) reported that the egg production in ladybirds was determined by the availability of a prey. The number of eggs laid by females of *A. cardoni* was positively correlated with, *A. gossypii* density (Omkar & Kumar, 2013). The prey density-dependent productivity was also observed in *Scymnus coccivora* Ayyar preying on cotton mealybug, *P. solenopsis* (Kumari, Suroshe, Kumar, Budhlakoti, & Yana, 2021). Egg production in treated *C. montrouzieri* females with oils and palizin was less affected than buprofezin, probably due to differences in the mode of action. Buprofezin can negatively affect egg formation in adult insects by preventing the production of the hormone prostaglandin (Ishaaya, Mendelson & Melamed-Madjar, 1988; Uchida, Isawa & Sugimoto, 1987). The adverse effect of buprofezin on the egg production has been reported by several researchers (Grafton-Cardwell & Gu, 2003; James, 2004). Buprofezin (0.1 and 0.2 a.i./liter) had a destructive effect on the egg formation of the predatory coccinellid, *Delphastus catalinae* Horn (Liu & Stansly, 2004). Penetration of an insecticide into the reproductive system of female and male adults, may cause malformations and affect eggs production (Retnakaran, Granett, & Ennis 1985; Mathew, Vijayalaxmi, & Rahiman, 1992; Carpenter & Chandler, 1994).

According to the results, the natural control effects of *C. montrouzieri* would be weakened by the application of buprofezin. But palizin had less adverse effect on functional and numerical responses of the ladybeetle, *C. montrouzieri*. It can be used in conjunction with *C. montrouzieri* in the integrated pest management of citrus scales. The oils were found to be effective against *P. citri* and according to the results, they had less adverse effects than buprofezin on *C. montrouzieri* female adults. So, they can be used as an alternative to the insecticide. But use of oils for control of scale bugs should be at the proper dose and time to avoid hazardous effects on the predator. Moreover, greenhouse and field based studies are needed to determine the compatibility of the insecticides with *C. montrouzieri* and effectiveness of exposed *C. montrouzieri* against scale bugs under more realistic conditions.

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Presence of Thysanoptera Species in the Urban Green Spaces of Iran: New Records Along with Illustrated Type Specimens

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ABSTRACT

We present a list of Thysanoptera species found in the Mashhad metropolitan region (north-east of Iran). A total of 24 sampling sites were set up on *Pinus mugo* Turra and *Platycladus orientalis* (L.), two ornamental trees important commonly found in urban areas, to better understand the fauna and distribution of thrips populations. Thysanoptera fauna consists of 13 species in eight genera, two subfamilies and three families, of which three have never been recorded in Razavi Khorasan province. In terms of species, the highest number of species belonged to the family Thripidae. The present study tentatively suggested that *P. mugo* and *P. orientalis* attract most species of adult thrips (Thysanoptera) but do not support reproduction. We examined and illustrated the types of eleven of currently accepted synonyms of *Haplothrips reuteri* (Karny, 1907), *H. subtilissimus* (Haliday, 1852), *Aptinothrips rufus* (Haliday, 1836), *Chirothrips africanus* Priesner, 1932, *C. manicatus* (Haliday, 1836), *Thrips atratus* Haliday, 1836, and *T. vulgatissimus* Haliday, 1836, together with information on distributions and diagnosis that are useful for recognising each species. The purpose of this study is to fill in the knowledge gaps regarding the distribution of Thysanoptera in the diverse urban green spaces (UGSs).

Key words: Fringed winged, ornamental, urban ecosystems, type, Iran, Palaearctic.

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INTRODUCTION

Urban green space is an important refuge for biodiversity in urban areas (Aronson et al, 2017). Insects are key components of urban ecological networks and are greatly impacted by anthropogenic activities (Sattler et al, 2011). However, the green patches in urban areas provide habitats for many insects and other arthropods, some of which have attained pest status (Robinson, 2005). Therefore, it is crucial to understand the relationship between urban green spaces and insect biodiversity. The process of urbanization in Iran has progressed sharply in recent decades, with urban areas expanding nearly ten-fold over the last 40 years, and a rapid trend that will be maintained in the coming 20 years. Mashhad, the second-largest city in Iran, is a typical example of urban development. Hence, understanding the biodiversity-urbanization relationships in Mashhad may provide a suitable model for other cities in Iran, and worldwide.

Thrips (Thysanoptera) comprise a hyperdiverse monophyletic clade of insects distributed worldwide (Buckman et al, 2012). Thysanopterans present a wide range of feeding strategies from phytophagy and mycophagy to predatory (Mound, 2004), making them a suitable model taxon to better understand the responses of insect herbivores and predators to gradients in urban vegetation structure and diversity. Thrips also provide important ecosystem services. For example, some the species such as *Frankliniella intonsa* (Trybom, 1895) and *Thrips tabaci* Lindeman, 1889 are indicators of changes in agroecosystems, climate changes and air pollution (Vasiliu-Oromulu et al, 2008). Some phytophagous thrips species are highly hosted plant-specific but sometimes exhibit remarkable host shifts (Mound, 1997). Polyphagous species sometimes are locally associated exclusively with a single host plant. Polyphagy and host range might be related to the availability of particular compounds in the host plants (Terry, 1997), or such thrips species might be unusually flexible in their feeding behaviour. The world fauna of Thysanoptera has +6,300 named species divided into nine extant families into two suborders, Tubulifera (one family) and Terebrantia (eight families and 90% of pest species) (Mound, 1997; Mound & Morris, 2007; ThripsWiki, 2021).

There are 270 distinct species of thrips from Iran (Mirab-Balou, 2018), but just a few species such as *Frankliniella occidentalis* Pergande, 1895 and *T. tabaci*, are known as ornamental plants pests in urban green spaces (Cloyd, 2009). Up to the present, there are just a few scattered studies conducted on thrips species associated with urban green spaces (i.e., in Poland by Czepiel, 2004; in China and Qatar by Mirab-Balou et al, 2014a, b), and a few common species of thrips in the global urban environment were mentioned by Robinson (2005). The current study is the first study devoted to documenting the biodiversity of thrips species in Mashhad's urban green spaces, an area where there is a scarcity of information about the species composition, ecological connections, and the number of thrips present.

MATERIAL AND METHODS

The specimens studied were obtained by field sampling in urban green spaces in northeastern Iran (Razavi Khorasan province; Fig. 1). Mashhad (36°18′N, 59°34′E) is

the second-largest city in Iran (with a population of 3.372 million in 2016) and occupies an area of 328 km^2. Mashhad has a typical concentric urban expansion pattern, and its green space accounted for 4% of the area covered. By random selection from a satellite map using Google Maps® and subsequent extensive field surveys in the study area, we identified 24 suitable green study patches (Fig. 1). The selected patches varied from 30 to 720 ha in size (Table 1). The vegetation was mainly composed of managed lawn mixed with some canopy species, woodland and shrubs (*Pinus mugo* Turra and *Platycladus orientalis* (L.)). Most of the patches were intensively managed, including frequent weed removal and tree care (see photograph in the lower left of Fig. 1). Methods used for collecting were hand-searching and beating sheets (Ng & Zaimi, 2018). Beating the plant parts, thrips were dislodged and landed on the cloth where they can easily be seen. Then we collected with an alcohol-soaked cotton swab and put it in a vial of alcohol. Samples were taken from leaves, branches and buds of *P. mugo* and *P. orientalis*. Samples collected were processed onto permanent microscopic slides in Canada Balsam, after maceration in 5% NaOH and dehydration through a series of alcohols of 70%, 80%, and 90% (Ng & Zaimi, 2018). These slides were then dried at 45 °C on a slide warmer, before being transferred into an oven for at least one month. All studied slides are deposited in the Entomology Museum of the Ferdowsi University of Mashhad, Iran (EMFUM).

Identifications and diagnoses were carried out with a differential interference contrast (DIC) microscope (Micros® MCX100) as indicated in the photomicrographs. Pictures of slide-mounted Bagnall's specimens photographed by an Olympus® BX63 microscope with phase contrast. The software running for image acquisition is cellSens Dimension 1.15, Core Version xv 3.14 (build 14760). Images were acquired digitally using Helicon Focus® software. These images are ©The Trustees of the Natural History Museum, London, and made available under Creative Commons License 4.0.

The Canadian specimen, *Taeniothrips lemanis* Treherne, 1924, was taken using a Leica® DM6B upright microscope equipped with a DMC4500 camera and Leica® Application Suite (LAS) X version 3.7.3. Each of the image stacks was taken using the software optimized number of steps based on the defined distance between in-focus vertical extremes of the specimen/structure in the field of view. Before imaging the specimen, white balancing, and configuration of the gamma correction and exposure were done manually to optimize the image quality. Images of the *Taeniothrips lemanis* Treherne, 1924 holotype (CNC1881689) are provided by B.M.T. Brunet at the Canadian National Collection of Insects, Arachnids and Nematodes, ©Her Majesty The Queen in Right of Canada, as represented by the Minister of Agriculture and Agri-Food, licensed under the Open Government License - Canada. All images were further processed using various minor adjustment levels in Adobe Photoshop® such as image cropping and rotation, adjustment of contrast and brightness levels, color saturation, and background enhancement. Morphological terminology follows Priesner (1948), Bailey (1951) and Bhatti (1988, 2006). Distributions in Iran were followed by Mirab-Balou et al, (2018). The list of Iranian provinces follows the Standard ISO 3166 and acronyms consisting of two

capital letters are used (Table 2). The distribution maps of all species were generated using SimpleMappr (Shorthouse, 2010). Acronyms for depositories are listed below:

CNC	Canadian National Collection, Ottawa, Canada
DU	Depository Unknown
EMFUM	Entomology Museum of Ferdowsi University of Mashhad, Mashhad, Iran
FNHM	Finnish Natural History Museum, Helsinki, Finland
NHMUK	Natural History Museum, London, United Kingdom
SNM	Statens Naturhistoriske Museum, Copenhagen, Denmark

Fig. 1. Map of the study area within Iran (created by Maphill). The location of 24 green patches surveyed in this study is shown as the location icon. The sample photograph in the lower left shows the vegetated and managed conditions of most surveyed green patches.

Table 1. List of 24 urban green patches of Mashhad metropolis in the northeast of Iran.

Green Space Name	No. species	Green Space Name	No. species
Mashhad River Park	2	Alandasht Garden	3
Chehel Baze Park	1	Bahar Park	2
Khorshid Kouhestan Park (First site)	3	Vahdat Park	2
Khorshid Kouhestan Park (Second site)	1	Aftab Boostan (First site)	8
Pardis Qaem Park	4	Aftab Boostan (Second site)	5
Astane Qods Garden (First site)	5	Behesht Park	3
Astane Qods Garden (Second site)	1	Basij Park	2
Pansado-Davazdah Dastgah Park	3	Eram Park	4
Mellat Park	6	Nosrat Park	1
Malek Abad Garden (First site)	5	Raja Park	4
Malek Abad Garden (Second site)	2	Karim Abad	1
Kooh Sangi Park	4	Qaleh Sakhteman	1

Table 2. The standard codes for provinces of Iran.

Province	Code	CD	Province	Code	CD
Alborz	AL	Central	Kordestan	KD	West
Ardebil	AR	North West	Lorestan	LO	West
Bushehr	BS	South	Markazi	MK	Central
Chaharmahal and Bakhtiari	CM	Central	Mazandaran	MN	North
East Azarbaijan	EA	North West	North Khorasan	KS	East
Esfahan	ES	Central	Qazvin	QZ	Central
Fars	FA	South	Qom	QM	Central
Guilan	GI	North	Razavi Khorasan	KV	East
Golestan	GO	North	Semnan	SM	Central
Hamadan	HD	West	Sistan and Baluchestan	SB	East
Hormozgan	HG	South	South Khorasan	KJ	East
Ilam	IL	West	Tehran	TE	Central
Kerman	KE	South East	West Azarbaijan	WA	North West
Kermanshah	KH	West	Yazd	YA	Central
Khuzestan	KZ	South West	Zanjan	ZA	North West
Kohgiluyeh and Boyer-Ahmad	KB	South	**31 Provinces**	**IR - Western Asia**	

CD: Cardinal Directions.

RESULTS

Our current study is one of several that focus on the taxonomy, distribution and species composition of Thrips species in Iran's urban green spaces, thereby providing a list of three families, two subfamilies, eight genera and thirteen species in the 24 urban green patches. In terms of species, the highest number of species belonged to the family Thripidae. *Aeolothrips intermedius* Bagnall, 1934, *Chirothrips africanus* Priesner, 1932 and *Thrips vulgatissimus* Haliday, 1836 are new records for Razavi Khorasan province.

Taxonomic treatment

Class Insecta Linnaeus, 1758

Order Thysanoptera Haliday, 1836

Suborder Terebrantia Haliday, 1836

Family Aeolothripidae Uzel, 1895

Genus *Aeolothrips* Haliday, 1836

***Aeolothrips intermedius* Bagnall, 1934** (Figs. 2, 15A, 16)

Aeolothrips fasciata adusta Uzel, 1895.

Aeolothrips fasciata aptera Karny, 1910.

Aeolothrips intermedius Bagnall, 1934.

Aeolothrips pontica Derbeneva, 1966.

Type information. Lectotype ♀ - Zurich, Switzerland (NHMUK).

Material examined. LECTOTYPE, SWITZERLAND • Zurich; Uetliberg; 2500 ft; female; July 1925; ex. *Medicago sativa* L.; Bagnall Coll.; B. M. Reg. No. 1932–339; drawer 51:1:4; no. barcode: #010153697; other catalog numbers: NHMUK:ecatalogue:6077695; occurrence ID: 74fe984d-dd94-4f4f-acf1-13e9374078f0 (NHMUK; Fig. 15A). IRAN • Razavi Khorasan province; Mashhad County; 01.VI.2017; 3 females and 2 males; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. Female: Length of fore wing distal dark band at anterior margin 1.0-1.5 times the length of pale area between the dark bands; spermatheca with 7-9 rather strong spiniform processes on sides of median groove. **Male:** Abdominal tergites IV-V with well-developed dorsal tubercles.

Geographical distribution. Oriental (China, India, Pakistan); **Palaearctic** (Albania, Austria, Bulgaria, China, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iran (AL, EA, ES, FA, GI, GO, HD, IL, KB, KD, KE, KH, KZ, MK, QZ, TE, WA, YA, ZA; Fig. 15), Italy, Latvia, Lithuania, Montenegro, Netherlands, North Africa, Norway, Poland, Portugal, Romania, Russia, Serbia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom).

Note. This species is recorded for the first time in Razavi Khorasan province, Iran.

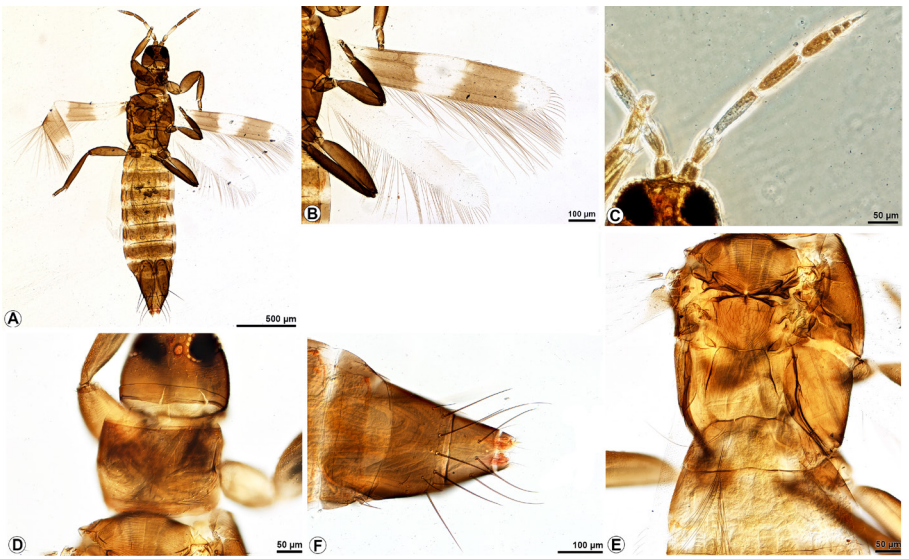

Fig. 2. *Aeolothrips intermedius* Bagnall, 1934. Lectotype, female (©NHMUK, #010153697). **A.** Habitus, ventral view. **B.** Wings, meso femur/tibia and meta femur/tibia. **C.** Antenna. **D.** Head, pronotum and fore femur, dorsal view. **E.** Mesonotum, metanotum and abdominal tergites I-II, dorsal view. **F.** Abdominal tergites VIII-X, dorsal view.

Family Phlaeothripidae Uzel, 1895

Subfamily Phlaeothripinae Uzel, 1895

Genus *Haplothrips* Amoyet & Serville, 1843

Haplothrips caespitis Priesner, 1936 (Figs. 3, 15B, 17)

Haplothrips caespitis Priesner, 1936.

Type information. Paratype ♀ - Managil, Sudan (NHMUK).

Material examined. PARATYPE, SUDAN • Managil; female; 16.I.1930; ex. *Cymbopogon proximus* (Maharaib) at roots; H. B. Johnston 145; drawer 51:6:104; no. barcode: #012816889; other catalog numbers: NHMUK:ecatalogue:9239467; occurrence ID: 875cb389-6283-471d-93d0-39658a45891c (NHMUK; Fig. 15B). IRAN • Razavi Khorasan province; Mashhad County; 17.IX.2017; 2 females; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. Female: Distal cilia of forewing smooth; head with maxillary stylets retracted to eyes, scarcely one-tenth of head width apart medially; postocular setae acute, scarcely extending beyond posterior margin of eyes; pronotal major setae acute and short, epimeral and posteroangular setae longest but scarcely longer than antennal segment III; fore tarsal tooth minute; mesopresternum reduced to two triangles; forewing with five or six duplicated cilia; sub-basal setae arranged in a triangle, finely acute and shorter than epimeral setae; metanotum with one pair of setae medially; abdominal tergites VII and VIII with seven or eight minor setae medially; tergite IX setae S1 and S2 finely acute, about 0.6 times as long as tube; tube 1.8 times as long as basal width.

Geographical distribution. Afrotropical (Sudan); **Oriental** (Pakistan); **Palaearctic** (Austria, Czech Republic, Estonia, France, Germany, Hungary, Iran (HD, KV, KZ; Fig. 16), Italy, Poland, Romania).

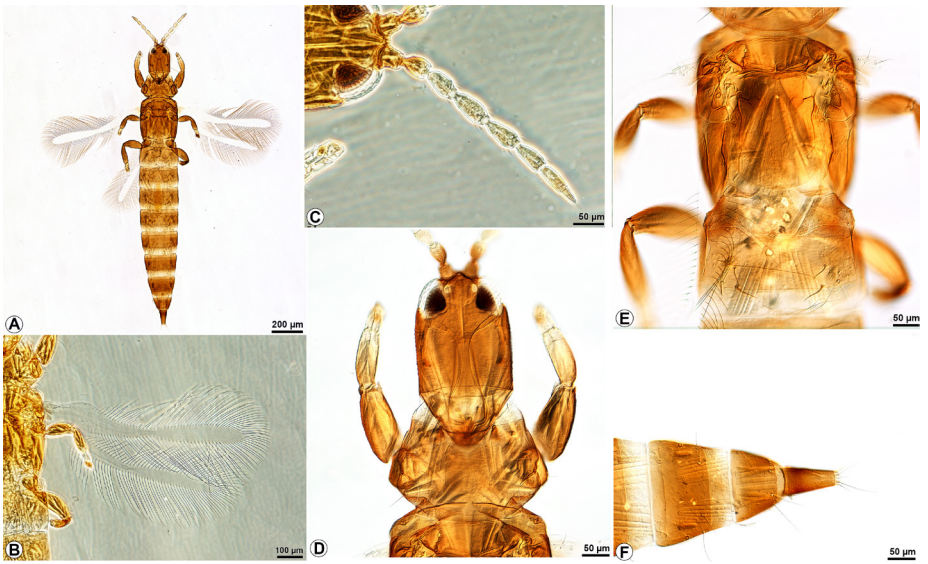

Fig. 3. *Haplothrips caespitis* Priesner, 1936. Paratype, female (©NHMUK, #012816889). **A.** Habitus, ventral view. **B.** Wings, mid and hind leg. **C.** Antenna. **D.** Head, pronotum, mesonotum and fore femur, dorsal view. **E.** Mesonotum, metanotum and abdominal tergites I, dorsal view. **F.** Abdominal tergites VIII-X, dorsal view.

***Haplothrips reuteri* (Karny, 1907)** (Figs. 4, 5, 15C-D, 18)

Haplothrips reuteri Karny, 1907.

Haplothrips tenuisetosus Bagnall, 1933.

Haplothrips satanas Bagnall, 1933.

Type information. Unknown type status - former Yugoslavia (DU).

Material examined. HOLOYPE, SUDAN • G. R. F.; Wad Madani; female; 7.IV.1932; ex. from flowers of *Dolicha lablab* (L.); identified as *Haplothrips tenuisetosus* Bagnall, 1933; BM 1933-628; A. P. G. Michal.; drawer 51:6:110; no. barcode: #012816890; other catalog numbers: NHMUK:ecatalogue:9239468; occurrence ID: fdd8a67f-3ddf-4242-bcda-e158c43cb929 (NHMUK; Fig. 15C). **HOLOTYPE**, FRANCE • Perpignan; female; VIII.1926; ex. *Centaurea solstitialis* L.; identified as *Haplothrips satanas* Bagnall, 1933; Bagnall Coll.; B. M. Reg. No. 1932-339; drawer 51:6:110; no. barcode: #012816891; other catalog numbers: NHMUK:ecatalogue:9239470; occurrence ID: f9266cf9-e26b-41f9-b03b-155a602156c2 (NHMUK; Fig. 15D). IRAN • Razavi Khorasan province; Mashhad County; 24.III.2017; 4 females and 2 males; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. Female: Distal cilia of forewing with surface rough; head with maxillary stylets 0.6-0.7 of head width apart, retracted to postocular setae; postocular setae pointed, long, extending beyond posterior margin of eyes; antennal segment III with two sensoria; pronotum with five pairs of finely pointed major setae, anteromarginal

setae more than twice as long as discal setae but shorter than anteroangulars; tergite VII with two campaniform sensilla not close to each other, and three to six micro setae present on tergites VII and VIII. Tergite IX setae S1, S2 and S3 acute, S1 long, a little shorter than tube, sub- equal to S2, S1 on other tergites pointed or finely pointed. **Male:** Pseudovirga bifid at tip, bearing two small appendages at apex.

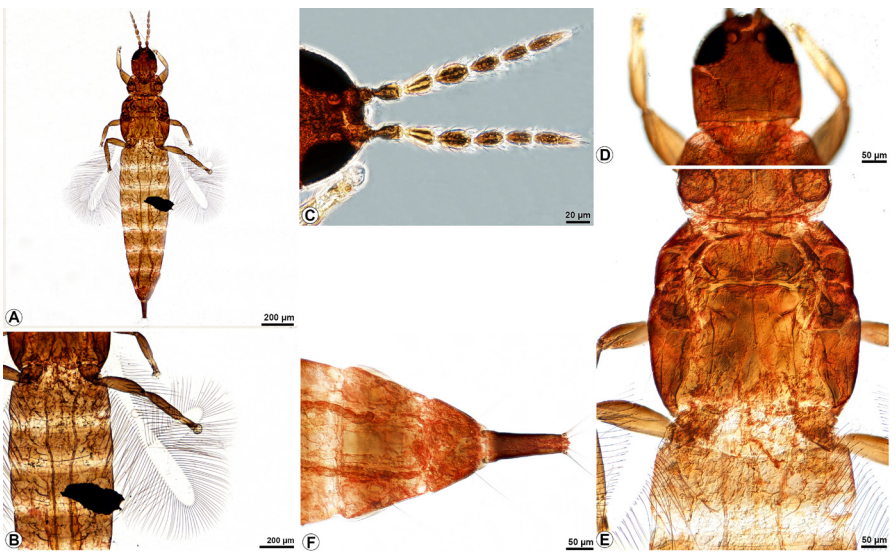

Fig. 4. *Haplothrips tenuisetosus* Bagnall, 1933. Holotype, female (©NHMUK, #012816890) [synonym of *Haplothrips reuteri* (Karny)]. **A.** Habitus, ventral view. **B.** Wings and hind leg. **C.** Antenna. **D.** Head, pronotum and fore femur/tibia, dorsal view. **E.** Pronotum, mesonotum, metanotum and abdominal tergites I, dorsal view. **F.** Abdominal tergites VIII-X, dorsal view.

Geographical distribution. Afrotropical (Sudan, Yemen); **Nearctic** (North America); **Oriental** (China, India, Pakistan,); **Palaearctic** (Albania, Austria, Bulgaria, China, Egypt, former Yugoslavia, France, Greece, Iran (AL, EA, ES, FA, GO, KE, KJ, KS, KV, KZ, MN, QZ, TE, ZA; Fig. 17), Lithuania, Mongolia, Qatar, Romania, Russia, Siberia, Slovenia, Spain, Turkey, Ukraine).

Notes. We examined the female holotype of *Haplothrips tenuisetosus* Bagnall, 1933 and a male holotype of *Haplothrips satanas* Bagnall, 1933 (NHMUK), which are currently synonyms of *Haplothrips reuteri* (Karny, 1907).

***Haplothrips subtilissimus* (Haliday, 1852)** (Figs. 6, 19)

Haplothrips subtilissimus Haliday, 1852.

Phloeothrips pallicornis Reuter, 1879.

Cryptothrips ovivorus Vasiliev, 1922.

Haplothrips atricornis Priesner, 1925.

Haplothrips inoptata Priesner, 1925.

Type information. Unknown type status - England (DU).

Fig. 5. *Haplothrips satanas* Bagnall, 1933. Holotype, female (©NHMUK, #012816891) [synonym of *Haplothrips reuteri* (Karny)]. **A.** Habitus, ventral view. **B.** Wings, mid and hind leg. **C.** Antenna. **D.** Head, pronotum and fore femur/tibia, dorsal view. **E.** Pronotum, mesonotum, metanotum and abdominal tergites I, dorsal view. **F.** Abdominal tergites VIII-X, dorsal view.

Fig. 6. *Phloeothrips pallicornis* Reuter, 1879. Syntype, female (©FNHM, #GV.33087) [synonym of *Haplothrips subtilissimus* (Haliday)]. **A.** Habitus, dorsal view. **B.** Head, pronotum and pro femur, dorsal view. **C.** Fore wing and abdominal tergites V-X, dorsal view. **D.** Antenna. **E.** Syntype labels.

Material examined. SYNTYPE, FINLAND • Ab Turku; Ispoinen; ykj: 6710:3240; Reuter Odo Morannal leg.; female; http://id.luomus.fi/GV.33087 (square label, with a QR barcode; printed); Mus. Zool.

H: fors, Spec. typ. No. 6032; identified as *Phloeothrips pallicornis* Reuter, 1879; Photographed 2020, Pekka Malinen (FNHM). IRAN • Razavi Khorasan province; Mashhad County; 31.V.2017; 2 females and 2 males; collected on *P. mugo* and *P. orientalis*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. Female: Antennae 8-segmented, segment III with one small sense cone, IV with 4 similar sense cones; VIII short; head longer than wide; maxillary stylets less than 0.5 of head width apart, retracted to postocular setae, maxillary bridge complete; postocular setae with blunt to weakly capitate apices, scarcely 0.7 as long as dorsal length of compound eyes; pronotum with 5 pairs of major setae with blunt to weakly capitate apices; epimeral sutures complete; prosternal basantra present, mesopresternum complete; fore tarsus without a tooth; fore wing constricted medially, with 8-12 duplicated cilia; sub-basal setae S1 and S2 capitate, S3 almost pointed. Tergite IX setae all acute, about 0.5 as long as tube. **Male:** With no pore plate on sternite VIII; fore tarsal tooth present; tergite IX setae S2 short and stout.

Geographical distribution. Nearctic (North America); **Oriental** (China); **Palaearctic** (Albania, Austria, Bulgaria, China, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Iran (FA, HD, KV, TE; Fig. 18), Italy, Japan, Latvia, Netherland, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, Ukraine, United Kingdom).

Notes. We examined the female syntype of *Phloeothrips pallicornis* Reuter, 1879 (FNHM), which is the current synonym of *Haplothrips subtilissimus* (Haliday, 1852).

Haplothrips tritici (Kurdjumov, 1912) (Fig. 20)

Haplothrips tritici Kurdjumov, 1912.

Haplothrips paluster Priesner, 1922.

Haplothrips cerealis Priesner, 1939.

Type information. Unknown type status - Russia (DU).

Material examined. IRAN • Razavi Khorasan province; Mashhad County; 22.IV.2017; 3 females; collected on *P. mugo* and *P. orientalis*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. ***Female:*** Head with postocular setae pointed, rarely weakly blunt, long, extending beyond posterior margin of eyes but sometimes shorter and not extending so far; maxillary bridge about 0.3-0.4 of head width, usually retracted to postocular setae; antennal segment III with two sensoria; pronotum with five pairs of major setae, anteromarginals pointed, usually twice as long as discal setae and smaller than anteroangulars, mesopresternum eroded medially; tergite IX setae S1 usually blunt, sometimes weakly blunt or finely pointed.

Geographical distribution. Australasian (Australia); **Nearctic** (North America); **Oriental** (China); **Palaearctic** (Albania, Algeria, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, China, Croatia, Czech Republic, Finland, France, Germany, Hungary, Iran (AL, CM, EA, ES, FA, GO, IL, KB, KD, KE, KH, KV, KZ, LO, MK, MN, QM, QZ, SM, TE, WA, YA, ZA; Fig. 19), Iraq, Italy, Korea, Lithuania, Macedonia, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia, Siberia, Slovakia, Slovenia, Spain, Sweden, Turkey, Ukraine).

Family Thripidae Stevens, 1829

Subfamily Thripinae Stevens, 1829

Genus *Aptinothrips* Haliday, 1836

***Aptinothrips rufus* (Haliday, 1836)** (Figs. 7, 21)

Thrips (*Aptinothrips*) *rufus* Haliday, 1836.
Thrips (*Aptinothrips*) *nitidulus* Haliday, 1836.
Aptinothrips rufa connaticornis Uzel, 1895.
Uzelliella lubbocki Bagnall, 1908.
Aptinothrips intermedius Priesner, 1920.
Aptinothrips groenlandica Richter, 1928.

Type information. Unknown type status - England (DU, probably lost).

Fig. 7. *Thrips (Aptinothrips) nitidulus* Haliday, 1836. Holotype, female (©SNM) [synonym of *Aptinothrips rufus* (Haliday)]. **A.** Habitus, lateral view. **B.** Voucher specimen (centre) with data label (left side). **C.** Data label (right side).

Material examined. HOLOTYPE, ENGLAND • West Greenland; Kujalleq; Igaliku-Fjord; female; 21.VIII.1989; W. Lindley; identified as *Thrips* (*Aptinothrips*) *nitidulus* Haliday, 1836 and *A. groenlandica* Richter (SNM). IRAN • Razavi Khorasan province; Mashhad County; 04.V.2017; 5 females; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. ***Female:*** Antennae 6-segmented; antennal segment VI shaded with brown; segment VI large resulting from fusion of 3 segments, VI twice as long as V; head elongate

with vertex weakly reticulate; pronotum weakly trapezoidal, without long setae; Meso and metanota transverse; prosternal basantra with no setae; ferna almost continuous medially; meso and metafurca without spinula; tergite IX posteromedian pair of setae short; tergites and sternites without craspedum and weakly reticulate; tarsi 1-segmented. **Male:** sternites without pore plates; tergite IX with 2 pairs of stout thorn-like setae.

Geographical distribution. Australasian (Australia); **Nearctic** (North America); **Neotropical** (Costa Rica); **Oriental** (China); **Palaearctic** (Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, China, Croatia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Iran (AL, EA, GO, HD, IL, KE, KD, KH, KS, KV, QZ, TE, WA, ZA; Fig. 20), Ireland, Italy, Lithuania, Netherlands, North Africa, Norway, Poland, Portugal, Qatar, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, United Kingdom).

Notes. We examined the female holotype of *Thrips* (*Aptinothrips*) *nitidulus* Haliday, 1836 (SNM), which is currently known as a synonym of *Aptinothrips rufus* (Haliday, 1836).

Genus *Chirothrips* Haliday, 1836

Chirothrips africanus Priesner, 1932 (Figs. 8, 15E, 22)

Chirothrips africanus Priesner, 1932.

Chirothrips aethiops Bagnall, 1932.

Chirothrips ramakrishnai Ananthakrishnan, 1957.

Type information. Unknown type status - Egypt (DU).

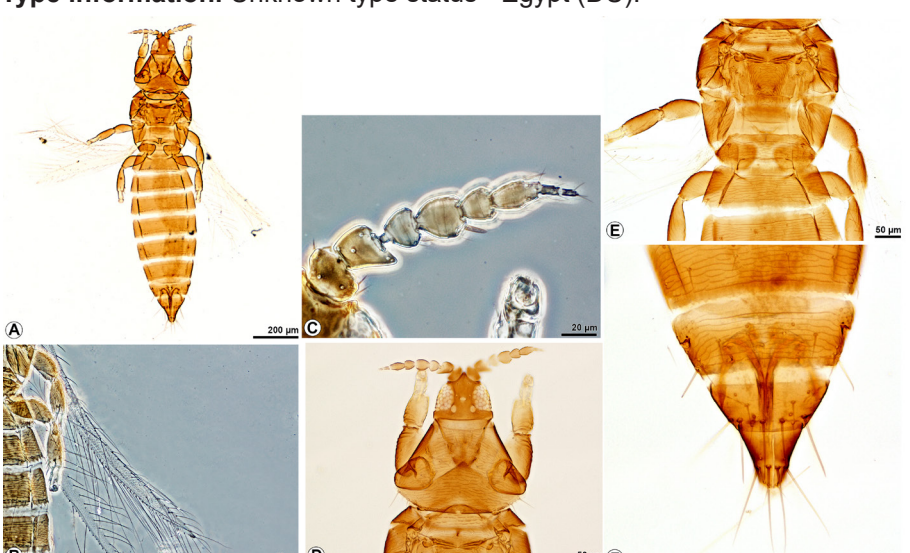

Fig. 8. *Chirothrips aethiops* Bagnall, 1932. Holotype, female (©NHMUK, #012816892) [synonym of *Chirothrips africanus* Priesner]. **A.** Habitus, ventral view. **B.** Wings, mid and hind leg. **C.** Antenna. **D.** Head, pronotum, mesonotum and fore leg, dorsal view. **E.** Mesonotum, metanotum and abdominal tergites I-III, dorsal view. **F.** Abdominal tergites VII-X, dorsal view.

Material examined. HOLOTYPE, SUDAN • G. R. F.; Wad Medani; female; 05.II.1931; ex. *Medicago sativa* L.; identified as *Chirothrips aethiops* Bagnall, 1932; BM 1933–628; W. P. L. Cameron; drawer 51:2:29; no. barcode: #012816892; other catalog numbers: NHMUK:ecatalogue:9239471; occurrence ID: 230aba34-3d7a-46b6-84b1-e4b0f401725f (NHMUK; Fig. 15E). IRAN • Razavi Khorasan province; Mashhad County; 01.IV.2017; 2 females; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. ***Female:*** Head prolongation in front of eyes scarcely 7; Ocellar region with 4 ante-ocellar setae; Antennal segment II outer margin almost straight.

Geographical distribution. Afrotropical (Ethiopia, Sudan, Yemen); **Nearctic** (North America); **Oriental** (India,); **Palaearctic** (Algeria, China, Cyprus, Egypt, Iran (GO, KV, SB; Fig. 21), Italy, Taiwan, Turkey, Uzbekistan).

Notes. This species is recorded for the first time in Razavi Khorasan province, Iran. We examined the holotype, a female specimen, of *Chirothrips aethiops* Bagnall, 1932 (NHMUK), which is the current synonym of *Chirothrips africanus* Priesner, 1932.

***Chirothrips manicatus* (Haliday, 1836)** (Figs. 9, 10, 11, 12, 15F-I, 23)

Thrips (*Chirothrips*) *manicatus* Haliday, 1836.

Thrips longipennis Burmeister, 1838.

Chirothrips antennatus Osborn, 1883.

Chirothrips manicata adusta Uzel, 1895.

Chirothrips fusca Coesfeld, 1898.

Chirothrips similis Bagnall, 1909.

Chirothrips manicata aptera Schille, 1912.

Chirothrips albicornis Priesner, 1926.

Chirothrips ammophilae Bagnall, 1927.

Chirothrips takahashii Moulton, 1928.

Chirothrips microptera Maltbaek, 1929.

Chirothrips brachyptera Maltbaek, 1929.

Chirothrips productus Bagnall, 1932.

Chirothrips ambulans Bagnall, 1932.

Chirothrips laingi Bagnall, 1932.

Chirothrips testacea Hukkinen, 1935.

Chirothrips bagnalli Hood, 1938.

Chirothrips longisetis Priesner, 1949.

Type information. Unknown type status - England (DU).

Material examined. LECTOTYPE, ENGLAND • Durham; Gillside; female; 1908; ex. 'grass'; identified as *Chirothrips similis* Bagnall, 1909; Bagnall Coll.; B. M. Reg. No. 1932–339; drawer 51:2:30; no. barcode: #012816893; other catalog numbers: NHMUK:ecatalogue:9239472; occurrence ID: f1db639a-a54f-44a6-b300-512acac31399 (NHMUK; Fig. 15F). **LECTOTYPE**, SPAIN • Galicia; female; identified as *Chirothrips ambulans* Bagnall, 1932; BM 1932–339; drawer 51:2:30; no. barcode: #012816894; other catalog numbers: NHMUK:ecatalogue:9239473; occurrence ID: 2a9b8ad9-e71b-48b3-a05f-980cf0d90670 (NHMUK; Fig. 15G). **LECTOTYPE**, FRANCE • Plage de Hyères; female; IX.1927; ex. *Ammophila*; identified as

Chirothrips laingi Bagnall, 1932; Bagnall Coll.; B. M. Reg. No. 1932–339; drawer 51:2:30; no. barcode: #012816895; other catalog numbers: NHMUK:ecatalogue:9239474; occurrence ID: a7dfbdf0-3f35-4e75-bda8-0bc50080dc46 (NHMUK; Fig. 15H). **SYNTYPE**, FRANCE • Plage de Hyères; female; IX.1927; ex. *Ammophila*; identified as *Chirothrips ammophilae* Bagnall, 1927; Bagnall Coll.; B. M. Reg. No. 1932–339; drawer 51:2:30; no. barcode: #012816896; other catalog numbers: NHMUK:ecatalogue:9239475; occurrence ID: 49388a51-b3c0-4d80-bc24-d5e625d74023 (NHMUK; Fig. 15I). IRAN • Razavi Khorasan province; Mashhad County; 22.VI.2017; 2 females and 4 males; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. Female: shaded forewings; head much smaller than the trapezoidal pronotum; antennae 8-segmented, segment I greatly enlarged, II strongly asymmetric; sense cones on III & IV simple; forewing slender and acute; mesothoracic furca well-developed with large lateral wing-like lobes; tergites with a simple antecostal ridge; sternite posterior margin with craspedum of small dark tubercles and submarginal row of similar smaller tubercles. **Male:** micropterous; tergites with strong lines of sculpture medially; sternites III–VII with small circular glandular area.

Geographical distribution. Australasian (Australia); **Nearctic** (North America); **Palaearctic** (Albania, Algeria, Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iran (AL, EA, GI, GO, HD, KE, KH, KZ, KS, QZ, KV, SB, YA; Fig. 22), Ireland, Italy, Korea, Latvia, Lithuania, Macedonia, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom).

Notes. We studied the female lectotype of *Chirothrips similis* Bagnall, 1909, female lectotype of *Chirothrips ambulans* Bagnall, 1932, female lectotype of *Chirothrips laingi* Bagnall, 1932, and female syntype of *Chirothrips ammophilae* Bagnall, 1927 (NHMUK), which all were synonymized under *Chirothrips manicatus* (Haliday, 1836).

Fig. 9. *Chirothrips similis* Bagnall, 1909. Lectotype, female (©NHMUK, #012816893) [synonym of *Chirothrips manicatus* (Haliday)]. **A.** Habitus, ventral view. **B.** Wings, mid and hind leg. **C.** Antenna. **D.** Head, pronotum and fore leg, dorsal view. **E.** Pronotum, mesonotum, metanotum and abdominal tergites I-II, dorsal view. **F.** Abdominal tergites VII-X, dorsal view.

Fig. 10. *Chirothrips ambulans* Bagnall, 1932. Lectotype, female (©NHMUK, #012816894) [synonym of *Chirothrips manicatus* (Haliday)]. **A.** Habitus, ventral view. **B.** Antenna. **C.** Abdominal tergites VIII-X, dorsal view. **D.** Head, pronotum, mesonotum and fore leg, dorsal view. **E.** Mesonotum, metanotum and abdominal tergites I-III, dorsal view.

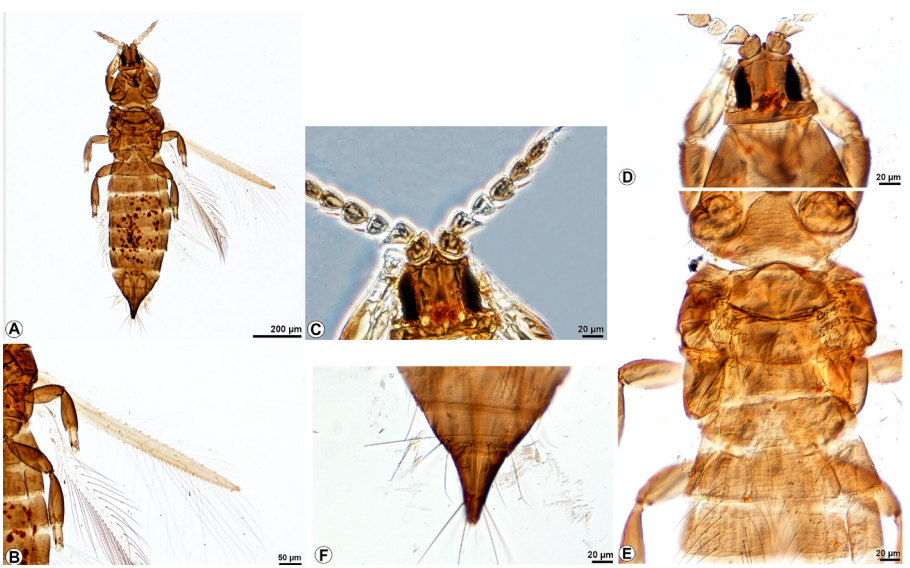

Fig. 11. *Chirothrips laingi* Bagnall, 1932. Lectotype, female (©NHMUK, #012816895) [synonym of *Chirothrips manicatus* (Haliday)]. **A.** Habitus, ventral view. **B.** Wings, mid and hind leg. **C.** Antenna. **D.** Head, pronotum and fore leg, dorsal view. **E.** Pronotum, mesonotum, metanotum and abdominal tergites I-III, dorsal view. **F.** Abdominal tergites VIII-X, dorsal view.

Fig. 12. *Chirothrips ammophilae* Bagnall, 1927. Syntype, female (©NHMUK, #012816896) [synonym of *Chirothrips manicatus* (Haliday)]. **A.** Habitus, ventral view. **B.** Wings, head, mesosoma, abdominal sternites, ventral view. **C.** Antenna. **D.** Head, pronotum and fore leg, dorsal view. **E.** Pronotum, mesonotum, metanotum and abdominal tergites I-III, dorsal view. **F.** Abdominal tergites VII-X, dorsal view.

Genus *Frankliniella* Karny 1910

***Frankliniella intonsa* (Trybom, 1895)** (Fig. 24)

Thrips intonsa Trybom, 1895.
Physopus vulgatissima nigropilosa Uzel, 1895.
Physopus vulgatissima fulvicornis Uzel, 1895.
Physopus vulgatissima albicornis Uzel, 1895.
Physopus vulgatissima adusta Uzel, 1895.
Thrips pallida Karny, 1907.
Physopus brevistylis Karny, 1908.
Frankliniella breviceps Bagnall, 1911.
Frankliniella vicina Karny, 1922.
Frankliniella intonsa maritima Priesner, 1925.
Frankliniella formosae Moulton, 1928.
Frankliniella formosae tricolor Moulton, 1928.
Frankliniella intonsa rufula Keler, 1936.
Frankliniella intonsa norashensis Yakhontov & Jurbanov, 1957.
Type information. Unknown type status - Finland (DU).

Material examined. IRAN • Razavi Khorasan province; Mashhad County; 08.V.2017; 8 females and 4 males; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. Female: antennal segments I & II, VI-VIII brown, III-V yellow with apex brown; forewing pale; head with 3 pairs of ocellar setae, pair III on anterior margins of ocellar triangle; major postocular setae less than half as long as ocellar setae III; antennae 8-segmented, III & IV with sense cone forked; pronotum with 2 pairs of long setae on anterior margin, 2 pairs of long posteroangulars, and one pair posteromedially; metanotum with median setae arising at anterior margin; campaniform sensilla usually absent; forewing with both longitudinal veins bearing complete row of setae tergites V-VIII each with a ctenidium laterally, on VIII anterolateral to spiracle; tergite VIII posterior margin with a comb of slender widely spaced microtrichia; sternites with no discal setae. **Male:** similar to female but paler, sternites III-VII with transverse glandular area; tergites IX-X with major setae sometimes stout.

Geographical distribution. Australasian (Australia); **Nearctic** (North America); **Oriental** (Indonesia, Malaysia, Philippines); **Palaearctic** (Albania, Algeria, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iran (AL, EA, ES, FA, GI, GO, HD, IL, KE, KD, KH, KS, KV, KZ, MK, MN, QZ, TE, WA, YA, ZA; Fig. 23), Italy, Japan, Korea, Latvia, Lithuania, Macedonia, Mongolia, Netherlands, Norway, Poland, Portugal, Qatar, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Taiwan, Turkey, Ukraine, United Kingdom).

Genus *Limothrips* Haliday, 1836

Limothrips angulicornis Jablonowski, 1894 (Fig. 25)

Limothrips angulicornis Jablonowski, 1894.

Limothrips setariae Jones, 1912.

Limothrips adusta Karny, 1914.

Type information: Unknown type status - Poland (DU).

Material examined. IRAN • Razavi Khorasan province; Mashhad County; 02.09.2017; 2 females and 1 male; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. Female: Major setae at apex of abdomen dark brown; head longer than wide, prolonged in front of eyes, without long setae; antennae 8-segmented, II with distal external margin produced into a tooth; III & IV with sense cone forked; VIII longer than VII; pronotum with one pair of prominent slender posteroangular setae; metanotal median setae small and arising behind anterior margin; forewing slender, first vein with 3 setae widely spaced on distal half, second vein with about 8 equally spaced setae; abdominal tergites with median pores situated close to posterior margin; VIII without a marginal comb but with one pair of stout setae laterally; IX with one pair of thorn-like setae laterally; X with one pair of stout thorn-like setae near apex. **Male:** Apterous, head without ocelli; tergite IX with median pair of setae very short and stout; sternites without glandular areas.

Geographical distribution. Australasian (Australia); **Nearctic** (North America); **Palaearctic** (Bulgaria, China, Cyprus, Hungary, Iran (AL, EA, FA, GO, HD, KE, KS, KV, KZ, MN, WA; Fig. 24), Italy, Macedonia, Netherlands, North Africa, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Turkey, Ukraine, United Kingdom).

Genus *Odontothrips* Amyot & Serville, 1843

***Odontothrips confusus* Priesner, 1926** (Fig. 26)

Odontothrips confusus Priesner, 1926.

Type information. Unknown type status - Hungary (DU).

Material examined: IRAN • Razavi Khorasan province; Mashhad County; 18.VI.2017; 5 females and 2 males; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. Female: Antennae 8-segmented; segments III-IV constricted to apex, each with forked sense cone; segment VI sense cone with enlarged oval base; dorsal apex of segment I with pair of setae; head wider than long; three pairs of ocellar setae present, pair III as long as distance between compound eyes, arising on or just outside anterior margins of ocellar triangle; pronotum with two pairs of long posteroangular setae; discal area with weak sculpture lines and about 10 setae; fore tibia apex with two small teeth on ventral/inner margin but without a stout seta at inner apex; mesonotum with paired anterior campaniform sensilla, median setae close to posterior margin; metanotum weakly reticulate; median setae long, arising at anterior margin; campaniform sensilla present or absent; fore wing first vein with setal row almost complete but with a small sub-apical gap; setal row complete on second vein; abdominal tergites with no ctenidia, segments II-III with sculpture lines medially, but none medially on remaining tergites; tergite VIII with posteromarginal comb broadly interrupted medially, with long slender microtrichia laterally; VIII with group of microtrichia anterolateral to spiracle; IX with 2 pairs of campaniform sensilla, X with long split; sternites without discal setae, S1 on VII arising in front of margin. **Male:** Tergite IX with pair of stout setae posterolaterally; sternites without pore plates; extruded genitalia with two pairs of stout endothecal spines supported by elongate canaliculi.

Geographical distribution. Palaearctic (Austria, Bulgaria, China, Croatia, Cyprus, Czech Republic, France, Germany, Greece, Hungary, Iran (AL, FA, GO, HD, KD, KE, KS, KV, LO, MK, QZ, WA, YA, ZA; Fig. 25), Italy, Lithuania, Poland, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom).

Genus *Thrips* Linnaeus, 1758

***Thrips atratus* Haliday, 1836** (Figs. 13, 15J, 27)

Thrips atratus Haliday, 1836.

Physothrips atrata adusta Uzel, 1895.

Ceratothrips britteni Bagnall, 1914.

Taeniothrips longicornis Priesner, 1926.

Type information. Holotype, ♀ - England (NHMUK).

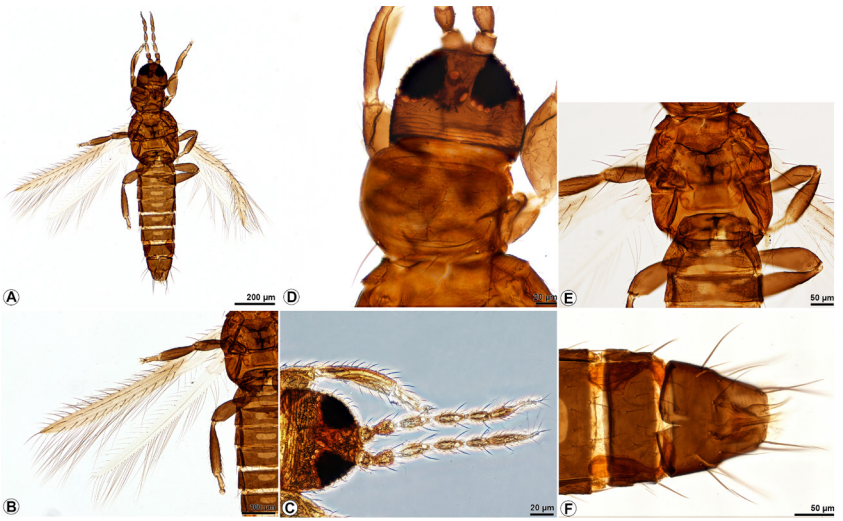

Fig. 13. *Ceratothrips britteni* Bagnall, 1914. Holotype, male (©NHMUK, #010153998) [synonym of *Thrips atratus* Haliday]. **A.** Habitus, ventral view. **B.** Wings, mid/hind leg, mesosoma, abdominal sternites, ventral view. **C.** Antenna and head. **D.** Head, pronotum and fore leg, dorsal view. **E.** Mesonotum, metanotum and abdominal tergites I-III, dorsal view. **F.** Abdominal tergites VIII-X, dorsal view.

Material examined. HOLOTYPE, ENGLAND • Cumbria Great Salkeld; male; 16.IX.1913; ex. *Scabiosa succisa*; identified as *Ceratothrips britteni* Bagnall, 1914; Bagnall Coll.; B. M. Reg. No. 1932-339; drawer 51:4:70; no. barcode: #010153998; other catalog numbers: NHMUK:ecatalogue:6181003; occurrence ID: 4789e205-8b02-41d2-ac77-f9902cff0abd (NHMUK; Fig. 15J). IRAN • Razavi Khorasan province; Mashhad County; 16.IX.2017; 2 females and 2 males; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. Female: Antennae 8-segmented; segments III-IV each with forked sense cone; head with cheeks convex, with 2 pairs of ocellar setae; pair III arising on anterior margins of, and slightly longer than side of, ocellar triangle; postocular setae pairs I & III well-developed but shorter than ocellar setae pair III, postocular setae pair II minute; pronotum anterior margin longest setae no more than 1.5 times as long as discal setae; with 2 pairs of long posteroangular setae; posterior margin with 3 (or 4) pairs; mesonotum with anterior campaniform sensilla, median setae arise well in front of posterior margin; metanotum with parallel lines of sculpture converging medially at posterior; median setae arising near anterior margin; campaniform sensilla present; fore wing first vein with 5 or more setae on distal half; second vein with complete row of about 14 setae; abdominal tergite II with 3 lateral marginal setae, V-VIII with paired ctenidia, on VIII posteromesad to spiracles; tergite VIII posteromarginal comb complete, microtrichia long and regular; pleurotergites each with 3-4 discal setae, posterior margin not dentate; tergite IX with 2 pairs of campaniform sensilla, X with median split; sternite II with few or no discal setae, III-VII with 8-20 discal setae in irregular double row, most numerous on posterior sternites; sternite VII setae S1 arise in front of margin. **Male:** tergite VIII without posteromarginal comb; tergite IX median setae slender; sternites III-VII with broadly transverse pore plate in front of discal setae, these pore plates progressively smaller on posterior sternites.

Geographical distribution. Nearctic (Canada); **Oriental** (China); **Palaearctic** (Albania, Belgium, Bosnia and Herzegovina, Bulgaria, China, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iran (FA, GO, HD, KB, KD, KE, KH, KS, KV, MK, MN, QZ, SB, TE, YA, ZA; Fig. 26), Italy, Ireland, Latvia, Lithuania, Macedonia, Moldova, Mongolia, Netherlands, North Africa, Norway, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom).

Notes. We examined the holotype, a male specimen, of *Ceratothrips britteni* Bagnall, 1914 (NHMUK), which is the current synonym of *Thrips atratus* Haliday, 1836.

***Thrips vulgatissimus* Haliday, 1836** (Figs. 14, 28)

Thrips vulgatissimus Haliday, 1836.

Physopus pallipennis Uzel, 1895.

Physopus pallipennis adusta Uzel, 1895.

Taeniothrips lemanis Treherne, 1924.

Taeniothrips gracilis Priesner, 1926.

Taeniothrips atricornis Priesner, 1926.

Taeniothrips americanus Moulton, 1929.

Physothrips gentianae Bagnall, 193.

Taeniothrips tahvanus Hukkinen, 1936.

Type information. Unknown type status - England (DU).

Fig. 14. *Taeniothrips lemanis* Treherne, 1924. Holotype, female (©CNC, #1881689) [synonym of *Thrips vulgatissimus* Haliday]. **A.** Habitus, ventral view. **B.** Wings, mid/hind leg, mesosoma, abdominal sternites, ventral view. **C.** Antenna [broken]. **D.** Head, pronotum and mesonotum, dorsal view. **E.** Mesonotum, metanotum and abdominal tergites I-III, dorsal view. **F.** Abdominal tergites VIII-X, dorsal view **G.** Holotype labels.

Fig. 15. Type labels of Thysanoptera species in ©NHMUK. **A.** *Aeolothrips intermedius* Bagnall, 1934. **B.** *Haplothrips caespitis* Priesner, 1936. **C.** *Haplothrips tenuisetosus* Bagnall, 1933. **D.** *Haplothrips satanas* Bagnall, 1933. **E.** *Chirothrips aethiops* Bagnall, 1932. **F.** *Chirothrips similis* Bagnall, 1909. **G.** *Chirothrips ambulans* Bagnall, 1932. **H.** *Chirothrips laingi* Bagnall, 1932. **I.** *Chirothrips ammophilae* Bagnall, 1927. **J.** *Ceratothrips britteni* Bagnall, 1914.

Material examined. HOLOTYPE, CANADA • British Colombia, Hatzic; female; 22.IV.1917; ex. off buds of *Acer macrophyllum*; identified as *Taeniothrips lemanis* Treherne, 1924; R.C. Treherne leg.; Type No. 679; specimen ID No. #CNC1881689 (CNC; Fig. 14G). IRAN • Razavi Khorasan province; Mashhad County; 05.IV.2017; 2 females and 4 males; collected on *P. mugo*; M. Heidari Latibari leg. (EMFUM).

Diagnosis. ***Female:*** Antennae 8-segmented, sense cone on III & IV small and forked head with no setae in front of fore ocellus, one pair of long setae on anterior margins of ocellar triangle; pronotum with 2 pairs of posteroangular setae; metanotum with transverse lines at anterior but longitudinal lines on posterior half; median setae not at anterior margin; forewing first vein with 3 or 4 setae on distal half; tergite II with 3 lateral marginal setae; V-VIII with ctenidia laterally, on VIII posteromesad of spiracle; posteromarginal comb on tergite VIII complete with slender microtrichia; sternites with transverse row of about 12 discal setae; pleurotergites with 2, 3 or 4 discal setae. **Male:** tergite VIII without posteromarginal comb; sternites III-VII with broadly transverse pore plate in front of discal setae.

Geographical distribution. Australasian (Australia); **Nearctic** (Canada); **Palaearctic** (Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iran (FA, HD, IL, KH, KS, KV, LO, MK, QZ, TE, ZA; Fig. 27), Ireland, Italy, Latvia, Macedonia, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom).

Notes. This species is recorded for the first time in Razavi Khorasan province, Iran. We examined the holotype of *Taeniothrips lemanis* Treherne, 1924 (CNC), a female specimen, which is the current synonym of *Thrips vulgatissimus* Haliday, 1836. The Canadian specimen is in pretty rough shape, however, with several appendages and the antennae separated from the remainder of the body. The body also appears to have broken/split in several locations.

DISCUSSION

Rapid urbanization is generally considered to be one of the main drivers of biodiversity loss, resulting in major local extinctions, decreases in native species diversity, changes in species composition and outbreaks of individual species (McKinney, 2002). Therefore, it is important to preserve green spaces within urban areas for insect biodiversity. Additionally, pest control is critical for protecting trees since trees are such an integral part of the urban ecosystem, however, this issue receives a great deal less attention than it deserves. In areas with urban green spaces, thysanopteran species are considered as one of the major pests (Robinson, 2005). Many thrips species are found in urban areas (e.g., Mirab-Balou et al, 2014a, b). But there have been no significant attempts yet to explore the Thysanoptera fauna in Iranian urban green spaces compared to other ecosystems (Fekrat & Manzari, 2014; Mirab-Balou et al, 2018). Only Mirab-Balou et al, (2015) have empirically studied Thysanoptera on ornamental plants in Iran. From urban green spaces in Romania, Vassiliu-Oromulu et al, (2009) recorded 20 species, and among them, *Frankliniella intonsa* was considered the most frequent species that matches our data. In urban green spaces of Hangzhou (Zhejiang Province in China), Mirab-Balou et al, (2014a) reported 26 species of thrips, and their results indicated that the fauna of thrips in urban ecosystems was quite diverse and abundant. During a recent report published by Mirab-Balou et al, (2014), nine new thrips were documented in the urban environments of Doha (Qatar), which were all new records for Qatari fauna. A total of four of the species with the greatest relevance to our present study were found in Qatar, including *Aptinothrips rufus*, *Chirothrips manicatus*, *Frankliniella intonsa*, and *Haplothrips reuteri*.

According to the number of species collected, Aeolothripidae has the lowest number of species (one), then followed by Phlaeothripidae (four) and Thripidae (eight) (Table 3). Based on the total number of specimens, *Aptinothrips rufus* and *Frankliniella intonsa* were the most abundant species in both urban and agricultural areas, which are also widespread throughout the world. Based on the results of the current research, it can be concluded that Thysanoptera species can be found in urban environments in varying densities. A study of the Thysanoptera fauna in Razavi Khorasan province has

been carried out by Fekrat & Manzari (2014) who found 45 species and 20 genera belonging to three families but have still been little studied and its systematics remains insufficient. This study increased the number of species to forty-eight.

We also found that the two species *Aeolothrips intermedius* and *Haplothrips subtilissimus* act as predators (Table 3), which could be effective biocontrol agents of different pests and therefore would potentially be relevant for biological control. The other species found in these surveys were phytophagous on many graminicolous and florivorous plants) (Table 3). All species have been collected from *Pinus mugo* and *Platycladus orientalis*, but neither are the exclusive hosts of these species. Our observation shows that no colonies built up on young or senescent needles of *P. mugo* and *P. orientalis*, and the absence of pre-adults has confirmed this proof. Hence, thrips in urban environments can only be considered casuals. We do not yet know the original host associations of these thrips. They may have host-shifted from other plants' leaves onto tree needles a long time ago, or they may have evolved as oligophages. Consequently, the presence of adults on a plant, even when they are in large numbers, does not necessarily indicate primary host associations with the plant (Mound, 2013). It may be worthwhile to pursue further studies to verify the original host associations.

Table 3. Associated thrips species with urban green spaces in northeast of Iran (Mashhad metropolis, Razavi Khorasan Province).

Family	Subfamily	Species	Host associations
Aeolothripidae	–	*Aeolothrips intermedius* Bagnall	Predatory (Facultative)
Phlaeothripidae	Phlaeothripinae	*Haplothrips caespitis* Priesner	Phytophagy (Graminicolous)
Phlaeothripidae	Phlaeothripinae	*Haplothrips reuteri* (Karny)	Phytophagy (Florivorous)
Phlaeothripidae	Phlaeothripinae	*Haplothrips subtilissimus* (Haliday)	Predatory
Phlaeothripidae	Phlaeothripinae	*Haplothrips tritici* (Kurdjumov)	Phytophagy (Graminicolous)
Thripidae	Thripinae	*Aptinothrips rufus* (Haliday)	Phytophagy (Graminicolous)
Thripidae	Thripinae	*Chirothrips africanus* Priesner	Phytophagy (Florivorous)
Thripidae	Thripinae	*Chirothrips manicatus* (Haliday)	Phytophagy (Florivorous)
Thripidae	Thripinae	*Frankliniella intonsa* (Trybom)	Phytophagy (Florivorous)
Thripidae	Thripinae	*Limothrips angulicornis* Jablonowski	Phytophagy (Graminicolous)
Thripidae	Thripinae	*Odontothrips confusus* Priesner	Phytophagy (Florivorous)
Thripidae	Thripinae	*Thrips atratus* Haliday	Phytophagy (Florivorous)
Thripidae	Thripinae	*Thrips vulgatissimus* Haliday	Phytophagy (Florivorous)

From the biogeographical point of view, all the thirteen species we collected are found in the Palaearctic region. *Haplothrips caespitis*, *H. reuteri* and *Chirothrips africanus* have already been recorded from the Afrotropical region (Mirab-Balou, 2018). We expect that more thrips are common to both Iran and the Afrotropical region, as share similar climatic and floral conditions (i.e., Heraty et al, 2019; Rakhshani et al, 2019; Ghafouri Moghaddam et al, 2018, 2019a, b, 2021, 2022; Derafshan et al, 2020,

2021; Moravvej et al, 2022). However, further field research is necessary to verify this hypothesis. Other regions documented six species (Australasian), ten species (Nearctic), nine species (Oriental) and one species (Neotropical). It is evident from distribution maps that eastern Iran has been largely ignored and sparsely sampled (Figs 15-27), and since many different species of insects are found in these areas (Heidari Latibari et al, 2016a, b, 2020, unpublished), thus, constant research is needed.

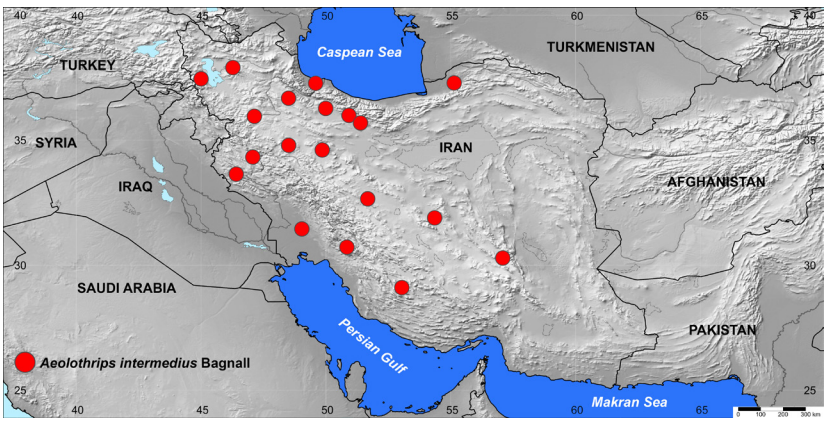

Fig. 16. Distribution map of *Aeolothrips intermedius* Bagnall, 1934 from Iran.

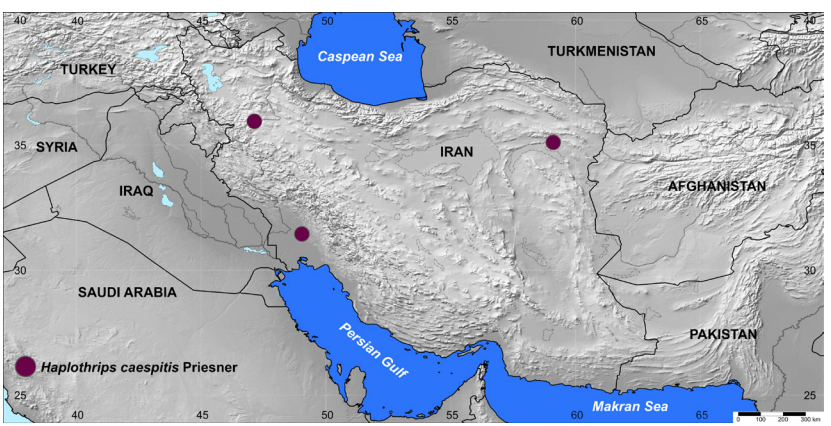

Fig. 17. Distribution map of *Haplothrips caespitis* Priesner, 1936 from Iran.

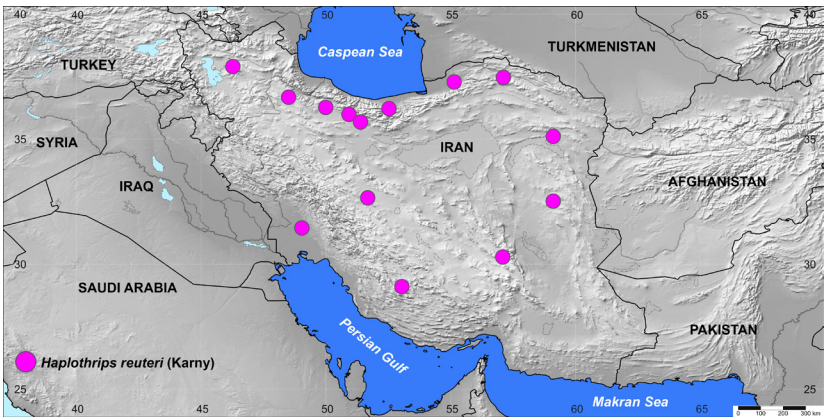

Fig. 18. Distribution map of *Haplothrips reuteri* (Karny, 1907) from Iran.

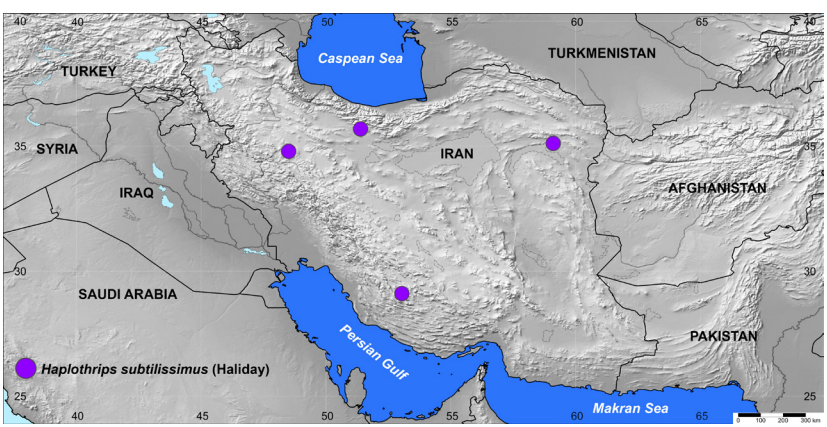

Fig. 19. Distribution map of *Haplothrips subtilissimus* (Haliday, 1852) from Iran.

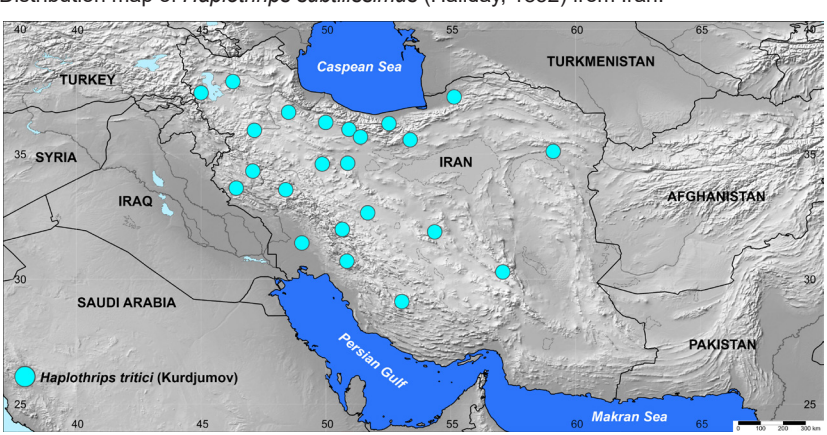

Fig. 20. Distribution map of *Haplothrips tritici* (Kurdjumov, 1912) from Iran.

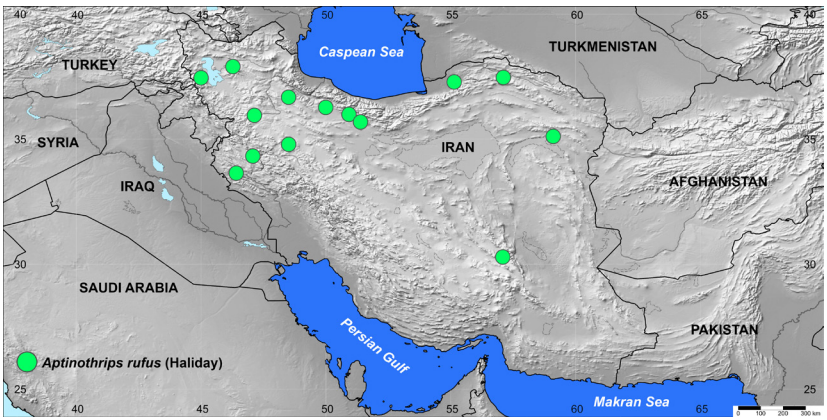

Fig. 21. Distribution map of *Aptinothrips rufus* (Haliday, 1836) from Iran.

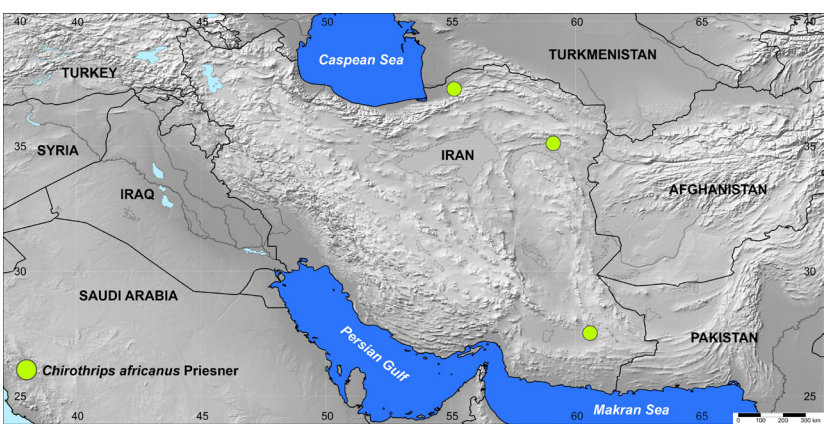

Fig. 22. Distribution map of *Chirothrips africanus* Priesner, 1932 from Iran.

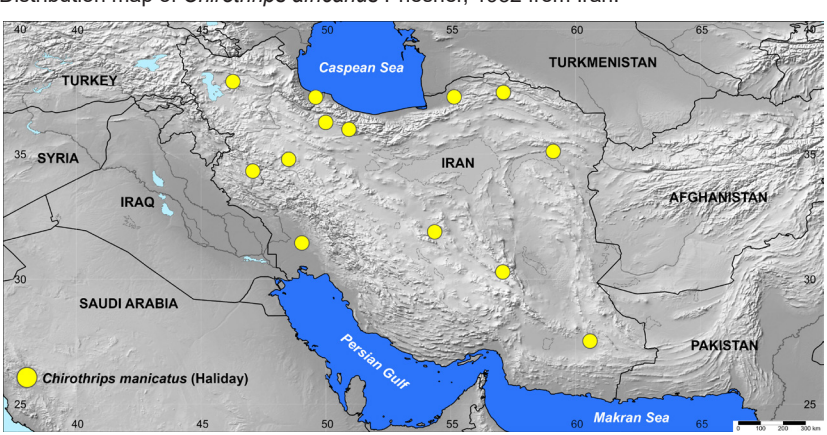

Fig. 23. Distribution map of *Chirothrips manicatus* (Haliday, 1836) from Iran.

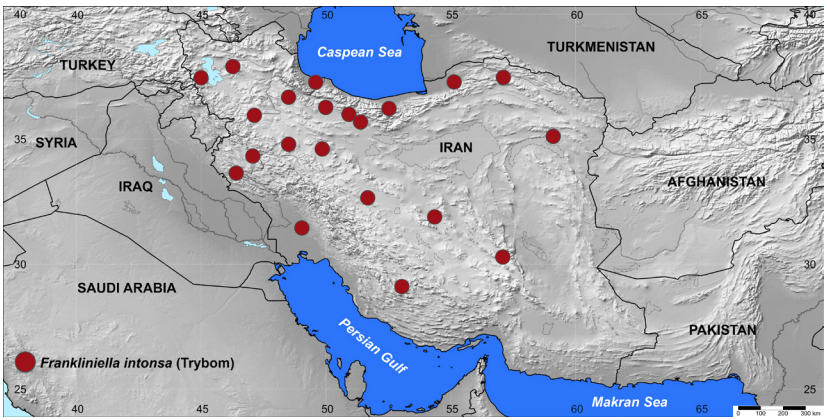

Fig. 24. Distribution map of *Frankliniella intonsa* (Trybom, 1895) from Iran.

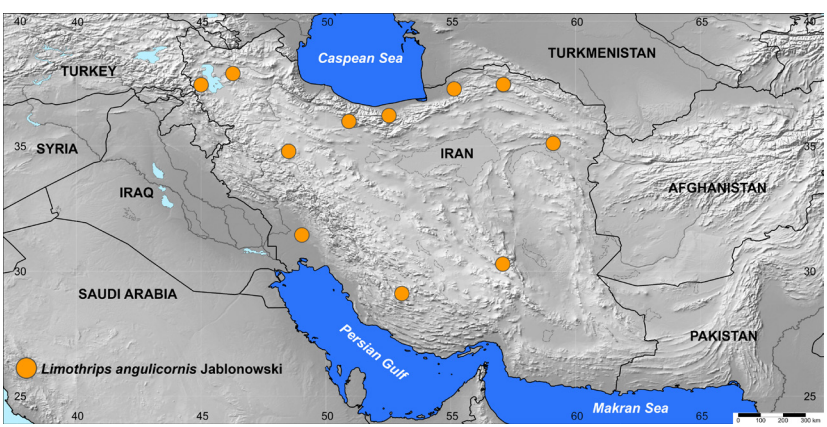

Fig. 25. Distribution map of *Limothrips angulicornis* Jablonowski, 1894 from Iran.

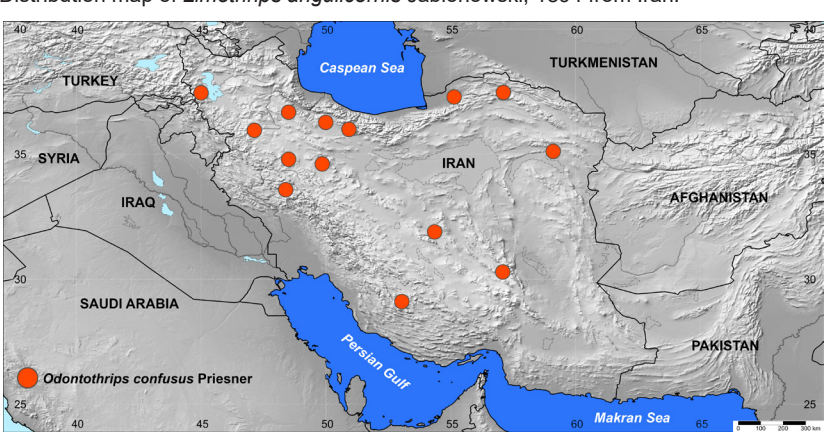

Fig. 26. Distribution map of *Odontothrips confusus* Priesner, 1926 from Iran.

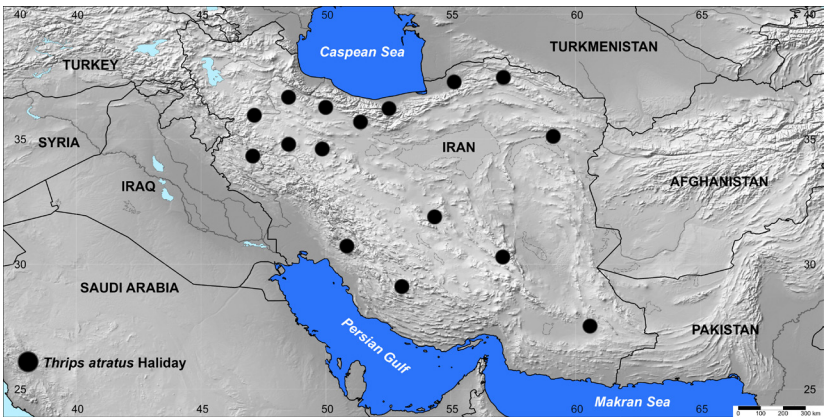

Fig. 27. Distribution map of *Thrips atratus* Haliday, 1836 from Iran.

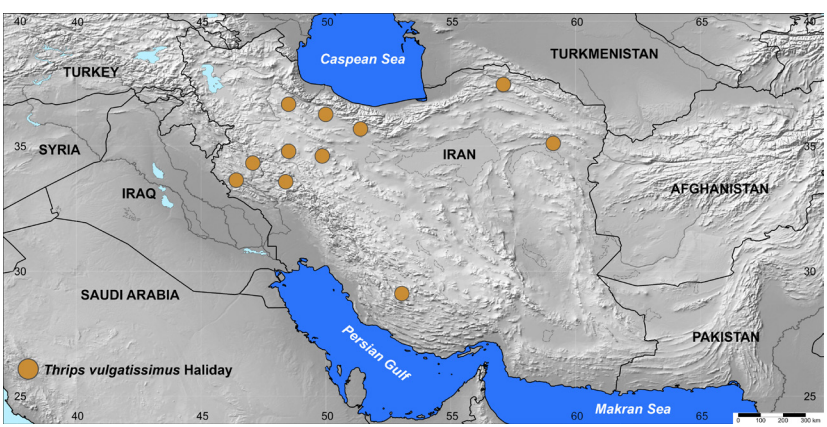

Fig. 28. Distribution map of *Thrips vulgatissimus* Haliday, 1836 from Iran.

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Cyptophania hirsuta, a New Psocid for Europe, Found in a Zoo Hothouse in the Netherlands (Psocodea: Lepidopsocidae)

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ABSTRACT

In a zoo hothouse in the Netherlands, the psocid *Cyptophania hirsuta* Banks, 1931 was sampled in 2019 and in 2021, indicating a population. These finds are remarkable, since the species was hitherto only known from Mexico and a few islands in Oceania. The introduction pathway is undoubtedly a consignment of tropical plants from central America that was used to furnish the hothouse.

Key words: alien species, import, tropical planting, Psocoptera.

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INTRODUCTION

The psocid fauna of Europe consists of a variety of naturally occurring, cryptogenic and alien species (Lienhard, 1998; Schneider, 2010). Several factors explain the large number of representatives in the latter two categories. Psocids are small and easily transported, especially because quite a few representatives are (semi) troglophilic, enabling them to thrive indoors and thus close to man. Psocids graze fungi and all sorts of (dry) organic substances, so they figure as pests of stored food products or may live amongst all sorts of goods and packaging material. Additionally, there are indications that these insects spread as aerial plankton over significant distances. A good deal of species have become widespread or cosmopolitan already long ago, their origins remaining largely unknown. This gap in our knowledge is enforced because of a general lack of faunistic and taxonomic studies on this insect group in most parts of the world.

In this article, *Cyptophania hirsuta* Banks, 1931 is reported as a new species for the fauna of the Netherlands and Europe. It is certainly an alien species here, since it was found in a zoo hothouse and the members of the genus live only in the tropics and subtropics and have never been seen in Europe before (Mockford & Wynne, 2013). The discovery is rather surprising, for *C. hirsuta* seems rare and was previously only known from Mexico and Oceania (Hawaii and Moorea in Polynesia and Fiji in Melanesia) (Banks, 1931; García Aldrete, 1985; Thornton, 1989; Johnson, Smith, Hopkins & Eades, 2021).

RESULTS

Cyptophania hirsuta Banks, 1931

Material examined: the Netherland, province of Drenthe, Emmen, Wildlands Adventure Zoo, tropical hothouse Jungola, 02.07.2019, 1 ♀ (Fig. 1), leg. J. Noordijk, col. Naturalis Biodiversity Center, Leiden (nr. RMNH.INS.1453293); ibidem, 11.02.2021, 1 ♀ (Fig. 2), leg. Bas Nijhof (after the photo was taken, the specimen was stored dry, withered and not stored in a collection).

The first specimen of *C. hirsuta* in Europe was collected in 2019 by the author while conducting an insect inventory of a zoo hothouse in Emmen, the Netherlands. The specimen was mounted and photographed (Fig. 1). Identification with European literature failed. Only after a second specimen was collected in 2021 by an entomologist working on biological control in the same hothouse and photographed (Fig. 2), the search for the identity continued. Because the majority of the planting in the hothouse was brought in from a nursery in Costa Rica (see below), the photos were send to two psocid specialists in the New World. Both dr. A.N. García Aldrete (Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico) and prof. dr. E.L. Mockford (School of Biological Science, Illinois State University, Illinois, USA) replied that *C. hirsuta* was the most likely candidate, and that several articles (Banks, 1931; Karny, 1932; Mockford & Wynne, 2013) should be consulted. With the aid of these sources, the identification became definite. *Cyptophania hirsuta* can be separated from its six congeners by its distinct venation in its forewing, whereby vein Sc arises from the wing base.

Banks (1931) provides a schematic drawing of the wing venation and habitus and Mockford & Wynne (2013) give an augmented description, including many detailed

drawings of body parts. In the present article, the first photographs of *C. hirsuta* are published (Figs. 1-2), which should make future identifications easier. The species has a highly characteristic appearance, with golden and dark patches of hairs over the complete body and elytriform wings encompassing the abdomen. Only females exist, since *C. hirsuta* is parthenogenetic (Mockford & Wynne, 2013).

Fig. 1. Female of *Cyptophania hirsuta*. The Netherlands, Emmen (province of Drenthe), Wildlands Adventure Zoo, tropical hothouse Jungola, collected on 2.07.2019. Photo: Theodoor Heijerman.

Fig. 2. Female of *Cyptophania hirsuta*. The Netherlands, Emmen (province of Drenthe), Wildlands Adventure Zoo, tropical hothouse Jungola, collected on 11.02.2021. Photo: Jan van Duinen.

DISCUSSION

Cyptophania hirsuta is neither known as a cosmopolitan nor a tramp species. In fact, its preceding documentation in only one country and three islands, would suggest it being rare. On the other hand, its presence on several remote islands indicates the species is easily introduced to new areas and it is very plausible that, due to the underrecording of Psocodea, its distribution area is larger than we know.

Very little is known on the biology of *C. hirsuta*, its biotopes are described as “on sugar cane”, “birds’ nest and dead leaves” and “foliage in woodland” (in Hawaii, Banks,

1931; Thornton, 1989; Mockford & Wynne, 2013) and “inner crater wall” (in Moorea, Thornton, 1989). The specimens from the Netherlands were gathered unconsciously during inventories, and no specific biotope in the zoo hothouse is known. Seen the current tropical distribution of the species, there seems very little chance of it establishing outdoors or even outside glasshouses in Europe.

There is a presumption on the origin of the population of *C. hirsuta* in the zoo hothouse. The zoo in Emmen was moved to a new location in the period 2013-2016. In the new hothouse, tropical plants from the former hothouses were planted, but circa 14.000 new tropical plants were imported from a large nursery in Costa Rica (Noordijk, van Veen, Groothuis & Schimmel 2019). Although Costa Rica is not bordering Mexico, where *C. hirsuta* is recorded, the option that this psocid was introduced with this consignment of plants seems likely. It also means that it is likely that *C. hirsuta* occurs in Costa Rica and maybe even in the other countries in Central America. Due to regular exchange of plants between zoos and other tropical hothouses, there is certainly a chance that *C. hirsuta* will also be found at other locations in Europe.

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Survey of Cynipid Gall Wasps (Hymenoptera, Cynipidae) in Serbia

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ABSTRACT

A list of 72 species of cynipid gall wasps that have been recorded to date in Serbia was compiled on the basis of studying cynipid fauna during the period from 2003 to 2020 and previously published data. The species *Andricus quercusramuli* (Linnaeus 1761) and *Pediaspis aceris* (Gmelin 1790) are recorded for the first time in the fauna of Serbia. The fauna of cynipid gall wasps in Serbia has still not been thoroughly studied. It is excepted that about 40 to 50 species new to the fauna will be found.

Keywords: Fauna, oak, *Quercus*, gall wasp, gall-inducing insect.

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INTRODUCTION

Cynipid gall wasps are small wasps (1.0 to 10.00 mm long) that create galls of different shape on trees, more rarely on herbaceous plants and shrubs. They belong to the family Cynipidae, which in addition to them also includes species that usually live as obligate inquilines in the galls of other cynipids (Melika, 2006a). With about 1400 species, cynipid gall wasps are the second largest group of gall-forming insects, after gall midges (Diptera, Cecidomyiidae) (Csóka, Stone, & Melika, 2005; Melika, 2006a). Their development involves the existence of only a sexual or an asexual generation in some species, while in others these two generations alternate cyclically. The galls they form in each generation, the host plant, the place where galls appear, and the shape, colour, structure, and size of its galls are characteristic of each species. That is why the determination can be done based on their characteristics, in addition to the morphological traits of adults. In some species, the galls they form are large and attractive and can be numbered among the most beautiful insect-created galls in the world (Melika, 2006a; Marković, 2014). Unfortunately, some species can cause damage by creating galls (Dobrosavljević, Kanjevac, & Marković, 2018; Avtzis, Melika, Matošević, & Coyle, 2018; Micik, Ozcankaya, Ocal, & Ipekdal, 2021).

Although data on the fauna of cynipid gall wasps in Serbia can be found in the studies of a number of authors (Langhoffer, 1915; Baudyš, 1928; Pal, 1983a, 1983b; Glavendekić & Mihajlović, 2004; Drekić, 2006; Drekić et al, 2020), it can be asserted that prior to 2006 these insects were not very much investigated in Serbia, except in Vojvodina (the northern part of Serbia), where 34 species have been recorded up to that time (Langhoffer, 1915; Pal, 1983a, 1983b). Their more detailed investigation was initiated that year. Some of the data that were then obtained have been published (Marković, 2014, 2015, 2018; Stojanović & Marković, 2016, 2017; Marković & Stojanović, 2017; Dobrosavljević et al, 2018; Drekić et al, 2020). Because the body of information about the cypinid of Serbia thereby grew considerably, the need arose to create the first summary of research conducted to date. Such a summary is also needed because it can be suspected that the number of species recorded up to now in Serbia as judged from the publications of Marković (2014, 2015, 2018) and Stojanović & Marković (2016, 2017) is inprecise. To verify whether this is the case and in view of all that has been said above, the first review of research on the fauna of cynipid gall wasps in Serbia is given in the present paper.

MATERIAL AND METHODS

The following review of research conducted to date on the cynipid gall wasps of Serbia is based on previously published data and material collected by us during the period from 2003 to 2020 at 27 localities (Table 1). Galls of cynipid gall wasps found at those localities were brought to the Laboratory of Entomology of Belgrade University´s Faculty of Forestry. There they were herbarized, identified, and deposited in the herbarium of zoocecidia of the Faculty of Forestry. They were identified by Č. Marković using published works of Ionesco (1957); Eady & Quinlan (1963); Ambrus (1974); Zerova,

Djakonchuk, & Ermolenko (1988); Csóka, Stone, & Melika (2005); Melika (2006a, 2006b); Acs, Melika, Pénzes, Pujade-Villar, & Stone (2007); and Melika et al (2010). The data obtained in this way and previously published data on the fauna of cynipid gall wasps in Serbia were used to form a database from which the list of recorded species was obtained. The names of species in it were coordinated with the names given by Melika (2006a, 2006b). For every species recorded in the course of our research, the generation whose galls were found in it is given, together with the host plants. The localities mentioned in the database were grouped according to the geographic entities (mountains) or administrative units (municipalities, urban areas) to which they belong.

Table 1. List of investigated localities.

Locality	Coordinates			Locality	Coordinates	
	N	E			N	E
Alibunar				Mt. Obla Glava		
Banatski Karlovci	45°2'	21°0'		Rujevica	43°32'	21°43'
Babušnica				Mt. Rtanj		
Zvonce, Vetren	42°55'	22°34'		Šarbanovac	43°41'	21°53'
Belgrade				Novi Sad		
Boljevci, Crni Lug forest	44°42'	20°12'		Čenej	45°22'	19°48'
Progar, Bojcin Forest	44°44'	20°8'		Pećinci		
Boljevac				Kupinovo, Kupinske Grede	44°42'	19°59'
Savinac	43°54'	22°2'		Obrež	44°44'	19°58'
Deliblato Sands				Ruma		
Girls´ Well	44°59'	20°57'		Hrtkovci	44°53'	19°45'
Kučevo				Platičevo	44°50'	19°46'
Mišljenovac	44°32'	21°34'		Šid		
Majdanpek				Morović	45°0'	19°13'
Debeli Lug	43°44'	22°6'		Sombor		
Debeli Lug, Felješana	44°20'	21°53'		Bukovac	45°43'	19°6'
Ravna Reka	44°26'	21°59'		Subotica		
Ujevac	44°25'	21°52'		Kelebia	46°9'	19°34'
Mt. Crni Vrh	44°8'	21°58'		Radanovac	46°7'	19°43'
Mt. Goč				**Žagubica**		
Brezna	43°33'	20°40'		Krepoljin, monastery Gornjak	44°15'	21°32'
Mt. Kalafat				Žagubica	44°11'	21°47'
Kamenički Vis	43°24'	21°57'				

RESULTS

Seventy species of cynipid gall wasps belonging to three tribes and 16 genera were recorded in previously published studies. Thirty-seven species from four tribes and 10 genera were registered in the course of our investigations. When all these data were entered in the database, the obtained list contained 72 species (Table 2).

Table 2. Cynipid gall wasps recorded in Serbia.

	Species	New finding/Reference
	Tribe Aylacini	
1	*Aulacidea hieracii* (Linnaeus 1758)	New finding of sexual generation: Deliblato Sands, Girls´ Well 7.6.2003. on *Hieracium umbellatum* L. Reference: Pal,1983a
2	*A. tragopogonis* (Thomson 1877)	Reference: Stojanović & Marković, 2017
3	*Aylax papaveris* (Perris 1839)	Reference: Stojanović & Marković, 2016
4	*Barbotinia oraniensis* (Barbotin 1964)	Reference: Stojanović & Marković, 2016; Marković & Stojanović, 2017
5	*Diastrophus mayri* Reinhard 1876	Reference: Marković & Stojanović, 2017
6	*D. rubi* (Bouché 1834)	Reference: Marković & Stojanović, 2017
7	*Liposthenes glechomae* (Linnaeus 1758)	Reference: Pal, 1983a
8	*Phanacis cichorii* (Kieffer 1909)	Reference: Stojanović & Marković, 2017
	Tribe Diplolepidini	
9	*Diplolepis eglanteriae* (Hartig 1840) (Fig.1a)	New finding of sexual generation: Alibunar, Banatski Karlovci 20.10.2008. on *Rosa* sp.; Boljevac, Savinac 21.10.2011. on *Rosa* sp.; Mt. Kalafat, Kamenički Vis 10.8.2012. on *Rosa* sp. Reference: Marković, 2014, 2015, 2018; Marković & Stojanović, 2017
10	*D. mayri* (Schlechtendal 1876)	Reference: Marković & Stojanović, 2017
11	*D. nervosa* (Curtis 1838) (Fig.1b)	Reference: Marković 2014, 2018; Marković & Stojanović, 2017
12	*D. rosae* (Linnaeus 1758) (Fig.1c)	New finding of sexual generation: Majdanpek, Debeli Lug 11.5.2013. on *Rosa* sp.; Mt. Crni Vrh 30.9.2011. on *Rosa* sp.; Mt. Kalafat, Kamenički Vis 29.8.2011., 10.8.2012. on *Rosa* sp.; Ruma, Hrtkovci 6.10.2011. on *Rosa* sp.; Žagubica, Krepoljin, monastery Gornjak 30.9.2011. on *Rosa* sp.; Žagubica 30.9.2011. on *Rosa* sp. Reference: Pal, 1983b; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017
13	*D. spinosissimae* (Giraud 1859) (Fig.1d,e)	Reference: Marković, 2014, 2018; Marković & Stojanović, 2017
	Tribe Pediaspidini	
14	*Pediaspis aceris* (Gmelin 1790) (Fig.1f)	New finding of sexual generation: Majdanpek, Debeli Lug, Felješana 21.5.2010. on *Acer pseudoplatanus* L.
	Tribe Cynipini	
15	*Andricus amblycerus* (Giraud 1859) (Fig.1g)	Reference: Marković, 2014, 2015, 2018; Marković & Stojanović, 2017
16	*A. aries* (Giraud 1859) (Fig.1h)	Reference: Marković & Stojanović, 2017; Drekić et al, 2020
17	*A. caliciformis* (Giraud 1859) (Fig.1i)	New finding of asexual generation: Belgrade, Progar, Bojcin Forest 15.7.2012. on *Q. robur*; Mt. Kalafat, Kamenički Vis 10.8.2012. on *Q. pubescens* Reference: Pal, 1983b; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017; Drekić et al, 2020

Table 2. Continued.

	Species	New finding/Reference
	Tribe Aylacini	
18	*A. callidoma* (Hartig 1841) (Fig.2a)	Reference: Marković, 2014, 2015; Marković & Stojanović, 2017
19	*A. caputmedusae* (Hartig 1843) (Fig.2b)	New finding of asexual generation: Majdanpek, Ujevac 14.7.2017. on *Q. petraea*; Žagubica 30.9.2011. on *Q. robur* Reference: Baudyš, 1928; Pal, 1983b; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017; Dobrosavljević et al, 2018
20	*A. conglomeratus* (Giraud 1859)	New finding of asexual generation: Pećinci, Obrež 14.2.2018. on *Q. robur* Reference: Pal, 1983b
21	*A. conificus* (Hartig 1843) (Fig.2c)	Reference: Marković, 2014; Marković & Stojanović, 2017
22	*A. coriarius* (Hartig 1843) (Fig.2d)	New finding of asexual generation: Mt. Kalafat, Kamenički Vis 10.8.2012. on *Q. pubescens* Reference: Pal, 1983b; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017; Drekić et al, 2020
23	*A. coronatus* (Giraud 1859) (Fig.2e)	New finding of asexual generation: Mt. Kalafat, Kamenički Vis 10.8.2012. on *Q. pubescens*; Mt. Rtanj, **Šarbanovac** 11.7.2018. **on** *Q. frainetto* Reference: Baudyš, 1928; Pal, 1983b; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017
24	*A. corruptrix* (Schlechtendal 1870)	Reference: Pal, 1983b
25	*A. curvator* Hartig 1840 (Fig.2f)	New finding of sexual generation: Belgrade, Progar, Bojcin Forest 15.7.2012. on *Q. robur*; Majdanpek, Debeli Lug, Felješana 21.5.2010. on *Q. petraea* Reference: Pal, 1983b; Glavendekić & Mihajlović, 2004; Marković, 2014, 2015; Marković & Stojanović, 2017; Drekić et al, 2020
26	*A. cydoniae* Giraud 1859 (Fig.2g)	Reference: Marković, 2014, 2015, 2018; Marković & Stojanović, 2017
27	*A. dentimitratus* (Rejto 1887)	Reference: Drekić et al, 2020
28	*A. foecundatrix* (Hartig 1840) (Fig.2h)	New finding of asexual generation: Belgrade, Boljevci, Crni Lug Forest 15.7.2012. on *Q. robur*; Progar, Bojcin Forest 15.7.2012. on *Q. robur*; Pećinci, Obrež 6.7.2017. on *Q. robur*; Pećinci, Kupinovo, Kupinske Grede 4.5.2017. on *Q. robur*; Ruma, Hrtkovci 6.10.2011., 9.7.2012. on *Q. robur*; Sombor, Bukovac 19.9.2011. on *Q. robur*; Subotica, Radanovac 19.9.2011. on *Q. robur* Reference: Pal, 1983b; Glavendekić & Mihajlović, 2004; Marković & Stojanović, 2017
29	*A. galeatus* (Giraud 1859) (Fig.2i)	Reference: Marković, 2014, 2018; Marković & Stojanović, 2017
30	*A. gallaeurnaeformis* (Boyer de Fonscolombe 1832) (Fig.3a)	Reference: Marković & Stojanović, 2017
31	*A. gemmeus* (Giraud 1859)	Reference: Pal, 1983b
32	*A. glutinosus* (Giraud 1859) (Fig.3b)	New finding of asexual generation: Babušnica, Zvonce, Vetren 10.4.2007. on *Q. petraea*; Mt. Crni Vrh 30.9.2011. on *Q. petraea* Reference: Marković, 2014, 2015; Drekić et al, 2020
33	*A. grossulariae* Giraud 1859 (Fig.3c,d)	Reference: Marković & Stojanović, 2017
34	*A. hartigi* (Hartig 1843) (Fig.3e)	Reference: Marković, 2014, 2015, 2018
35	*A. hungaricus* (Hartig 1843) (Fig.3f)	New finding of asexual generation: Ruma, Platičevo 19.2.2020. on *Q. robur*; Sombor, Bukovac 5.9.2014. on *Q. robur*; Subotica, Kelebija 19.9.2011. on *Q. robur* Reference: Pal, 1983b, Marković & Stojanović, 2017
36	*A. inflator* Hartig 1840 (Fig.3g,h)	New finding of sexual generation: Belgrade, Progar, Bojcin Forest 15.7.2012. on *Q. robur*; Novi Sad, Čenej 28.9.2012. on *Q. robur*; Ruma, Hrtkovci 9.7.2012. on *Q. robur* Reference: Marković & Stojanović, 2017; Marković, 2018

Table 2. Continued.

	Species	New finding/Reference
	Tribe Aylacini	
37	*A. kollari* (Hartig 1843) (Fig.3i)	New finding of asexual generation: Belgrade, Progar, Bojcin Forest 15.7.2012. on *Q. robur*; Majdanpek, Debeli Lug 16.6.2017. on *Q. petraea*; Majdanpek, Ravna Reka 15.7.2015. on *Q. petraea*; Novi Sad, Čenej 28.9.2012. on *Q. robur*; Pećinci, Kupinovo, Kupinske Grede 4.5.2017. on *Q. robur*; Pećinci, Obrež 6.7.2017. on *Q. robur*; Ruma, Platičevo 19.2.2020. on *Q. robur*; Subotica, Kelebija 19.9.2011. on *Q. robur* Reference: Langhoffer, 1915; Pal, 1983b; Glavendekić & Mihajlović, 2004; Marković, 2014; Marković & Stojanović, 2017; Dobrosavljević et al, 2018; Drekić et al, 2020
38	*A. lignicolus* (Hartig 1840) (Fig.4a)	New finding of asexual generation: Babušnica, Zvonce, Vetren 10.4.2007. on *Q. petraea*; Majdanpek, Debeli Lug, Felješana 21.5.2010. on *Q. petraea*; Mt. Crni Vrh 30.9.2011. on *Q. petraea*; Pećinci, Kupinovo, Kupinske Grede 4.5.2017. on *Q. robur* Reference: Pal, 1983b; Marković, 2014, 2015; Marković & Stojanović, 2017; Dobrosavljević et al, 2018; Drekić et al, 2020
39	*A. lucidus* (Hartig 1843) (Fig.4b)	New finding of asexual generation: Mt. Crni Vrh 30.9.2011. on *Q. petraea*; Mt. Kalafat, Kamenički Vis 10.8.2012. on *Q. pubescens*; Novi Sad, Čenej 28.9.2012. on *Q. robur*; Ruma, Hrtkovci 6.10.2011. on *Q. robur* Reference: Pal, 1983b; Marković, 2014; Marković & Stojanović, 2017; Drekić et al, 2020
40	*A. mitratus* (Mayr 1870) (Fig.4c)	New finding of asexual generation: Babušnica, Zvonce, Vetren 10.4.2007. on *Q. petraea*; Mt. Crni Vrh 30.9.2011. on *Q. petraea* Reference: Marković, 2014
41	*A. multiplicatus* Giraud 1859 (Fig.4d)	New finding of sexual generation: Ruma, Platičevo 19.2.2020. on *Q. cerris* Reference: Marković, 2014; Marković & Stojanović, 2017
42	*A. polycerus* (Giraud 1859) (Fig.4e)	New finding of asexual generation: Sombor, Bukovac 19.9.2011. on *Q. robur* Reference: Marković, 2015, 2018
43	*A. quercuscalicis* (Burgsdorf 1783) (Fig.4f)	New finding of asexual generation: Belgrade, Progar, Bojcin Forest 15.7.2012. on *Q. robur*; Novi Sad, Čenej 28.9.2012. on *Q. robur*; Ruma, Hrtkovci 6.10.2011. on *Q. robur*; Ruma, Platičevo 19.2.2020. on *Q. robur*; Sremska Mitrovica, Morović 8.10.2013. on *Q. robur*; Subotica, Kelebia 19.9.2011. on *Q. robur*; Žagubica 30.9.2011. on *Q. robur* Reference: Baudyš, 1928; Pal, 1983b; Marković, 2014; Marković & Stojanović, 2017; Dobrosavljević et al, 2018
44	*A. quercusradicis* (Fabricius 1798)	Reference: Marković, 2014; Marković & Stojanović, 2017
45	*A. quercusramuli* (Linnaeus, 1761) (Fig.4g)	New finding of sexual generation: Mt. Goč, Brezna 5.5.2008. on *Q. petraea*
46	*A. quercustozae* (Bosc 1792) (Fig.4h)	New finding of asexual generation: Mt. Kalafat, Kamenički Vis 29.8.2011. on *Q. pubescens*; Mt. Rtanj, **Šarbanovac** 26.7.2015. on *Q. frainetto*; Ruma, Hrtkovci 6.10.2011. on *Q. robur*; Žagubica 30.9.2011. on *Q. frainetto* Reference: Pal, 1983b; Marković, 2014; Marković & Stojanović, 2017
47	*A. seckendorffi* (Wachtl 1879) (Fig.4i)	Reference: Marković, 2015; Marković & Stojanović, 2017
48	*A. solitarius* (Boyer de Fonscolombe 1832) (Fig.5a)	New finding of asexual generation: Mt. Crni Vrh 30.9.2011. on *Q. petraea*; Mt. Kalafat, Kamenički Vis 29.8.2011. on *Q. petraea* and *Q. pubescens* Reference: Pal, 1983b; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017; Drekić et al, 2020
49	*A. stefanii* (Kieffer 1897) (Fig.5b)	New finding of asexual generation: Majdanpek, Debeli Lug 16.6.2017. on *Q. petraea*; Mt. Rtanj, **Šarbanovac** 11.7.2018. on *Q. pubescens* Reference: Dobrosavljević et al, 2018; Marković, 2018
50	*A. superfetationis* (Giraud 1859) (Fig.5c)	New finding of asexual generation: Novi Sad, Čenej 28.9.2012. on *Q. robur* Reference: Marković, 2018; Marković & Stojanović, 2017

Table 2. Continued.

	Species	New finding/Reference
	Tribe Aylacini	
51	*A. testaceipes* Hartig 1840	Reference: Pal, 1983b
52	*A. truncicolus* (Giraud 1859)	Reference: Pal, 1983b; Marković, 2015, 2018
53	*A. vindobonensis* Müllner 1901	Reference: Pal, 1983b
54	*Aphelonyx cerricola* (Giraud 1859) (Fig.5d)	New finding of asexual generation: Mt. Kalafat, Kamenički Vis 10.8.2012. on *Q. cerris*; **Žagubica, Krepoljin, monastery Gornjak** 30.9.2011. on *Q. cerris*; **Žagubica** 30.9.2011. on *Q. cerris* Reference: Marković, 2014, 2015, 2018; Marković & Stojanović, 2017
55	*Biorhiza pallida* (Olivier 1791) (Fig.5e)	New finding of sexual generation: Belgrade, Boljevci, Crni Lug Forest 15.7.2012. on *Q. robur*; Majdanpek, Debeli Lug 13.5.2013. on *Q. petraea*; Mt. Kalafat, Kamenički Vis 29.8.2011., 10.8.2012. on *Q. pubescens*; Pećinci, Kupinovo, Kupinske Grede 4.5.2017. on *Q. robur* Reference: Pal, 1983b; Glavendekić & Mihajlović, 2004; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017; Dobrosavljević et al, 2018; Drekić et al, 2020
56	*Callirhytis glandium* (Giraud 1859) (Fig.5f)	Reference: Marković & Stojanović, 2017
57	*Cynips agama* Hartig 1840	Reference: Baudyš, 1928; Pal, 1983b
58	*C. cornifex* Hartig 1843 (Fig.5g)	New finding of asexual generation: Mt. Kalafat, Kamenički Vis 29.8.2011., 10.8.2012. on *Q. pubescens* Reference: Marković, 2014, 2015, 2018
59	*C. longiventris* Hartig 1840 (Fig.5h)	New finding of asexual generation: Pećinci, Obrež 7.9.2017. on *Q. robur*; Ruma, Hrtkovci 6.10.2011. on *Q. robur*; Subotica, Kelebija 19.9.2011. on *Q. robur* Reference: Pal, 1983b; Glavendekić & Mihajlović, 2004; Marković & Stojanović, 2017
60	*C. quercus* (Fourcroy 1785) (Fig.5i)	New finding of asexual generation: Mt. Kalafat, Kamenički Vis 29.8.2011. on *Q. pubescens*; Mt. Rtanj, **Šarbanovac** 26.7.2015. on *Q. frainetto*; Ruma, Platičevo 19.2.2020. on *Q. frainetto* Reference: Pal, 1983b; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017; Dobrosavljević et al, 2018; Drekić et al, 2020
61	*C. quercusfolii* Linnaeus 1758 (Fig.6a)	New finding of asexual generation: Babušnica, Zvonce, Vetren 10.4.2007. on *Q. petraea*; Belgrade, Boljevci, Crni Lug Forest 15.7.2012. on *Q. robur*; Novi Sad, Čenej on *Q. robur*; Pećinci, Obrež on *Q. robur*; Ruma, Hrtkovci 6.10.2011. on *Q. robur*; Ruma, Platičevo 19.2.2020. on *Q. robur*; Žagubica 30.9.2011. on *Q. robur* Reference: Pal, 1983b; Glavendekić & Mihajlović, 2004; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017; Drekić et al, 2020
62	*Dryocosmus cerriphilus* (Giraud 1859) (Fig.6b,c)	Reference: Marković, 2014, 2015, 2018; Marković & Stojanović, 2017
63	*D. nitidus* (Giraud 1859) (Fig.6d)	New finding of asexual generation: Mt. Obla Glava, Rujevica 4.11.2008. on *Q. cerris* Reference: Marković & Stojanović, 2017
64	*Neuroterus albipes* (Schenck 1863)	Reference: Glavendekić & Mihajlović, 2004; Drekić et al, 2020
65	*N. anthracinus* (Curtis 1838) (Fig.6e)	New finding of asexual generation: Mt. Crni Vrh 30.9.2011. on *Q. petraea*; Mt. Rtanj, **Šarbanovac** 26.7.2015. on *Q. frainetto*; Novi Sad, Čenej 19.9.2011., 28.9.2012. on *Q. robur*; Ruma, Hrtkovci 6.10.2011. on *Q. robur*; Sombor, Bukovac 19.9.2011. on *Q. robur*; Subotica, Kelebija 19.9.2011. on *Q. robur*; Subotica, Radanovac 19.9.2011. on *Q. robur*; Žagubica 30.9.2011. on *Q. frainetto* Reference: Baudyš, 1928; Pal, 1983b; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017; Dobrosavljević et al, 2018; Drekić et al, 2020
66	*N. minutulus* Giraud 1859	Reference: Pal, 1983b

Table 2. Continued.

	Species	New finding/Reference
	Tribe Aylacini	
67	*N. numismalis* (Geoffroy in Fourcroy 1785) (Fig.6f)	New finding of sexual generation: Belgrade, Boljevci, Crni Lug Forest 15.7.2012. on *Q. robur*; Progar, Bojcin Forest 15.7.2012. on *Q. robur*; Kučevo, Mišljenovac 30.9.2011. on *Q. robur*; Novi Sad, Čenej 19.9.2011., 28.9.2012. on *Q. robur*; Pećinci, Obrež 6.7.2017., 8.8.2017. on *Q. robur*; Ruma, Hrtkovci 6.10.2011., 9.7.2012. on *Q. robur*; Sombor, Bukovac on 19.9.2011. *Q. robur*; Subotica, Kelebija 19.9.2011. on *Q. robur*; Subotica, Radanovac 19.9.2011. on *Q. robur* Reference: Pal, 1983b; Glavendekić & Mihajlović, 2004; Marković & Stojanović, 2017; Drekić et al, 2020
68	*N. quercusbaccarum* (Linnaeus 1758) (Fig.6g,h,i)	New finding of sexual generation: Pećinci, Kupinovo, Kupinske Grede 4.5.2017. on *Q. robur*; Mt. Kalafat, Kamenički Vis 29.8.2011., 10.8.2012. on *Q. pubescens*; Ruma, Hrtkovci 6.10.2011., 9.7.2012. on *Q. robur*; Majdanpek, Debeli Lug 11.5.2013. on *Q. petraea*. New finding of asexual generation: Belgrade, Boljevci, Crni Lug Forest 15.7.2012. on *Q. robur*; Progar, Bojcin Forest 15.7.2012. on *Q. robur*; Kučevo, Mišljenovac 30.9.2011. on *Q. robur*; Mt. Crni Vrh 30.9.2011. on *Q. petraea*; Novi Sad, Čenej 19.9.2011., 28.9.2012. on *Q. robur*; Pećinci, Obrež 8.8.2017. on *Q. robur*; Rtanj, Šarbanovac 11.7.2018. on *Q. frainetto*; Ruma, Platičevo 19.2.2020. on *Q. frainetto*; Sombor, Bukovac 19.9.2011. on *Q. robur*; Subotica, Kelebija 19.9.2011. on *Q. robur*; Subotica, Radanovac 19.9.2011. on *Q. robur*; Žagubica 30.9.2011. on *Q. frainetto* Reference: Pal, 1983b; Glavendekić & Mihajlović, 2004; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017; Dobrosavljević et al, 2018; Drekić, 2020
69	*N. saliens* (Kollar 1857)	Reference: Pal, 1983b
70	*N. tricolor* (Hartig 1841)	Reference: Baudyš, 1928
71	*Pseudoneuroterus macropterus* (Hartig 1843)	New finding of asexual generation: **Žagubica** 30.9.2011. on *Q. cerris* Reference: Pal, 1983b; Marković, 2014, 2015, 2018; Marković & Stojanović, 2017
72	*Trigonaspis megaptera* (Panzer 1801)	Reference: Pal, 1983b

DISCUSSION

As indicated above, it can be seen on the basis of all the assembled data that 72 species of cynipid gall wasps belonging to 4 tribes and 17 genera have been recorded to date in Serbia. The greatest number of them were found on *Q. robur* (31 species) and *Q. petraea* (31), followed by *Q. pubescens* (21), *Q. frainetto* (19), *Q. virgiliana* (14), *Q. cerris* (11), *Rosa* sp. (5), *C. intybus* (2), *P. rhoeas* (2), *A. pseudoplatanus* (1), *G. hirsute* (1), *H. sabaudum* (1), *P. argentea* (1), and *R. hirtus* (1). Among them, 70 species were found before (Langhoffer, 1915; Baudyš, 1928; Pal, 1983a, 1983b; Glavendekić & Mihajlović, 2004; Drekić, 2006; Marković, 2014, 2015, 2018; Stojanović & Marković, 2016, 2017; Markovic & Stojanović, 2017; Dobrosavljević et al, 2018; Drekić et al, 2020). In the course of our investigations, two species (*A. quercusramuli* and *P. aceris*) were recorded for the first time in the fauna of cynipid gall wasps of Serbia. Apart from them, five species new for the fauna of cynipid gall wasps of northern Serbia were found in our investigations (Ilić & Marković, 2015), together with five species new for the fauna of eastern Serbia and one species new for the fauna of western Serbia.

In terms of geography, apart from the region of Belgrade (46 species) (Marković & Stojanović, 2017), cynipid gall wasps have been most thoroughly investigated in the northern (46 species), eastern (44 species), and central (34 species) parts of Serbia. In southern and western Serbia, they have still been little studied. Cynipid gall wasps are mainly linked with oaks (Csóka et al, 2005). Since the same species of oaks are present in whole Serbia and because there is a great diversity of insects on them (Glavendekić, 2002; Marković & Stojanović, 2011, 2015, 2019; Marković, 2013; Marković, Stojanović, & Dobrosavljević, 2018; Marković, Dobrosavljević, Vujičić, & Cebeci, 2021; Stojanović, Jovanović, & Marković, 2018; Dobrosavljević, Marković, Marjanović, & Milanović, 2020), it is certain that many cynipid gall wasps will be registered in southern and western Serbia. The species that have been recorded to date in Serbia are taxa that can be expected in regions such as the ones in which they were found (Langhoffer, 1915; Ionescu, 1957; Ambrus, 1974; Kwas, 2012). The only exception is the species *B. oraniensis*, whose finding in Serbia is a surprise (Stojanović & Marković, 2016).

If the list of cynipid gall wasps in Serbia is compared with the list of species in neighbouring Hungary (Ionescu, 1957) and Romania (Ambrus, 1974), it can be concluded that it is realistic to expect that about 40 to 50 species new for the fauna of Serbia will be found. This applies especially to species that are linked with herbaceous plants, since cynipid gall wasps on them have been very little studied in Serbia (Pal, 1983a; Stojanović & Marković, 2016, 2017). Also, it is realistic to expect arrival of the harmful invasive species *Dryocosmus kuriphilus* Yasumatsu 1951 (Mick et al, 2021), which has already been recorded on the territory of neighbouring Hungary (Csóka, Wittmann, & Melika, 2009), Croatia (Matošević & Hrašović, 2013), and Bosnia-Herzegovina (Delalić, 2016).

One of the questions posed at the outset of these investigations was whether or not the number of cynipid gall wasps in Serbia given by Marković (2014, 2015, 2018) and by Stojanović & Marković (2016, 2017) is precise. Results of the present study show that it is not. According to the indicated authors, 84 species were found in Serbia at the time of their writing. That is 14 species more than the number of species (70) obtained by us in the present study on the basis of previously published data. This disagreement as to the number of species arose because: 1. The names of some species were synonyms; and 2. In one of the papers of Marković (2014), a mistake was made in stating that the number of species of cynipid gall wasps recorded up to that time was 67 instead of 57. Also, while speaking of mistakes, it should be noted that Marković and Stojanović (2017) erroneously wrote “sexual generation” instead of “asexual generation” for the species *C. glandium.*

On the basis of all that has been said, it can be concluded that with 72 species found to date, Serbia represents a region in which cynipid gall wasps have still not been thoroughly studied. For that to be achieved, about 40 to 50 species new to the fauna apparently need to be found and the regions of southern and western Serbia need to be investigated in greater detail.

Fig. 1. Galls of cynipid gall wasps. a. *Diplolepis eglanteriae* ♀♂; b. *D. nervosa* ♀♂; c. *D. rosae* ♀♂; d., e. *D. spinosissimae* ♀♂; f. *Pediaspis aceris* ♀♂; g. *Andricus amblycerus* ♀♀; h. *A. aries* ♀♀; i. *A. caliciformis* ♀♀ (sexual generation ♀♂; asexual generation ♀♀).

Fig. 2. Galls of cynipid gall wasps. a. ***Andricus callidoma*** ♀♀; b. *A. caputmedusae* ♀♀; c. *A. conificus* ♀♀; d. *A. coriarius* ♀♀; e. *A. coronatus* ♀♀; f. *A. curvator* ♀♂; g. *A. cydoniae* ♀♂; h. *A. foecundatrix* ♀♀; i. *A. galeatus* ♀♀.

Fig. 3. Galls of cynipid gall wasps. a. ***Andricus gallaeurnaeformis*** ♀♀; b. *A. glutinosus* ♀♀; c., d. *A. grossulariae*: c. ♀♂, d. ♀♀; e. *A. hartigi* ♀♀; f. *A. hungaricus* ♀♀; g., h. *A. inflator*: g. ♀♂, h. ♀♀; i. *A. kollari* ♀♀.

Fig. 4. Galls of cynipid gall wasps. a. ***Andricus*** *lignicolus* ♀♀; b. *A. lucidus* ♀♀; c. *A. mitratus* ♀♀; d. *A. multiplicatus* ♀♂; e. *A. polycerus* ♀♀; f. *A. quercuscalicis* ♀♀; g.*A. quercusramuli* ♀♂; h. *A. quercustozae* ♀♀; i. *A. seckendorffi* ♀♀.

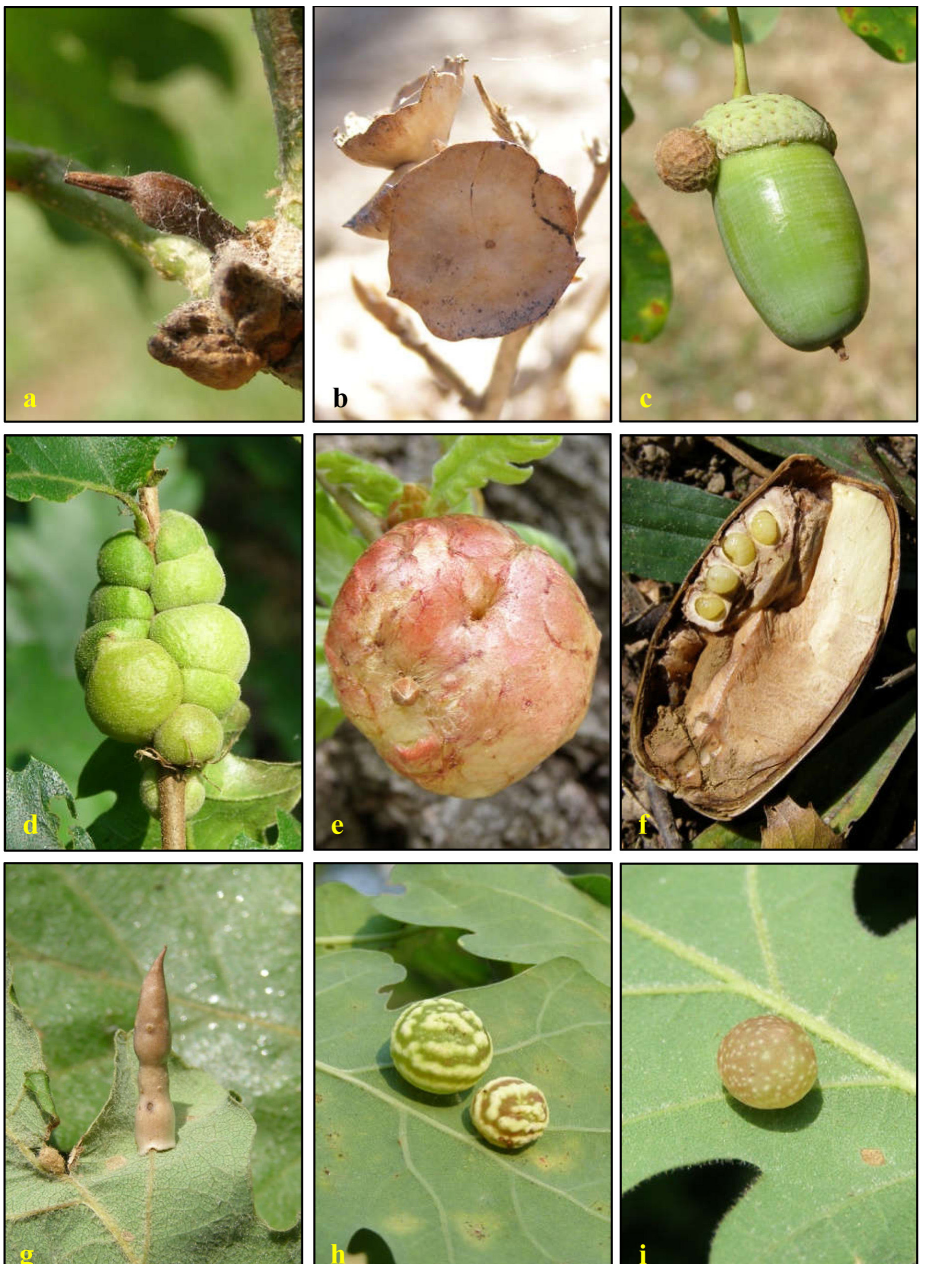

Fig. 5. Galls of cynipid gall wasps.a. *Andricus solitarius* ♀♀; b. *A. stefanii* ♀♀; c. *A. superfetationis* ♀♀; d. *Aphelonyx cerricola* ♀♀; e. *Biorhiza pallida* ♀♂; f. *Callirhytis glandium* ♀♀; g. *Cynips cornifex* ♀♀; h. *C. longiventris* ♀♀; i. *C. quercus* ♀♀.

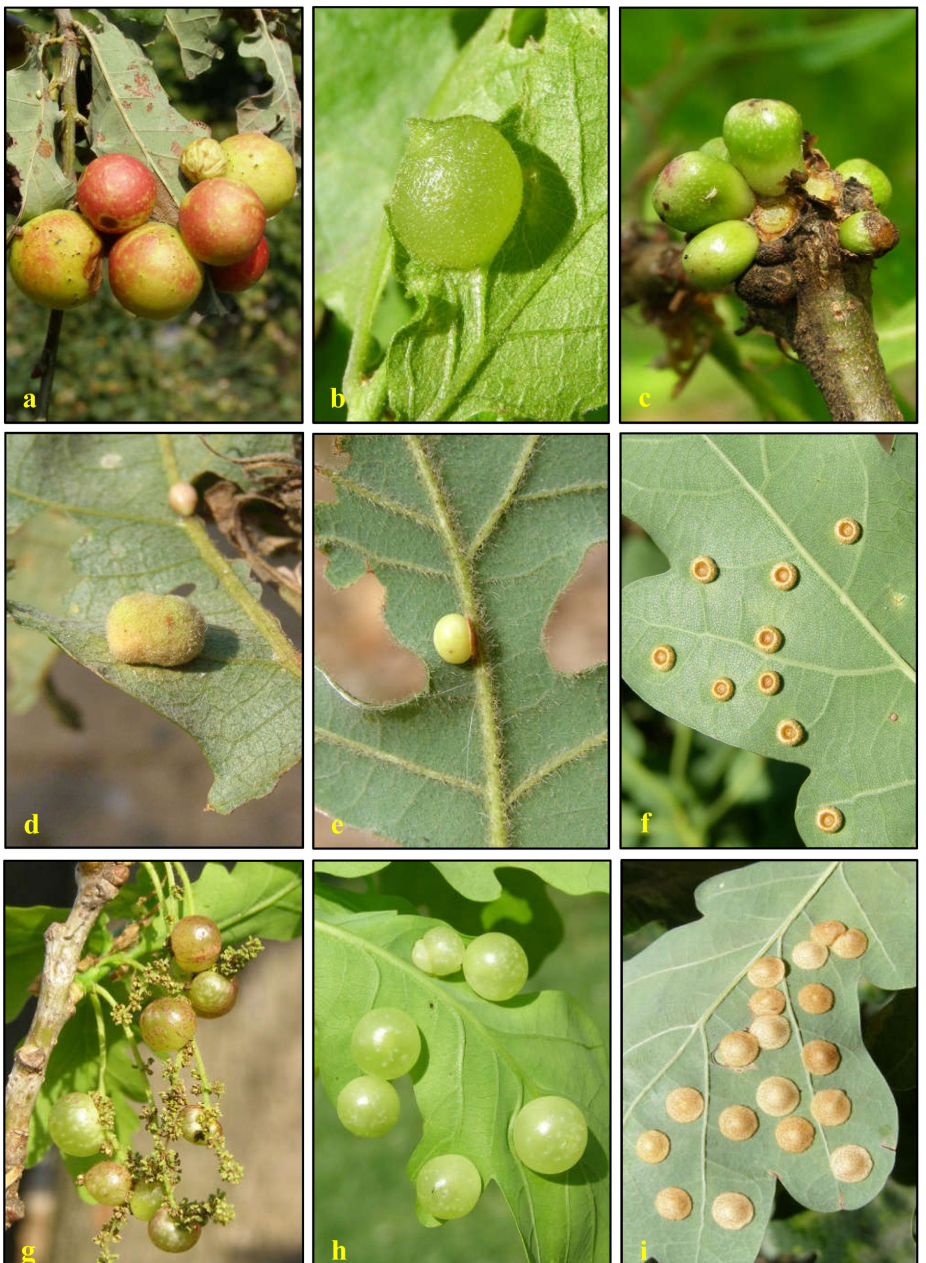

Fig. 6. Galls of cynipid gall wasps.a. *Cynips quercusfolii* ♀♀; b., c. *Dryocosmus cerriphilus*: b. ♀♂, c. ♀♀; d. *D. nitidus* ♀♀; e. *Neuroterus anthracinus* ♀♀; f. *N. numismalis* ♀♂; g., h. i. *N. quercusbaccarum*: g., h. ♀♂, i. ♀♀.

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Phosphine Resistance in Turkish Populations of *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae)

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ABSTRACT

In this study, the status of phosphine resistance in *Sitophilus oryzae* (L.,1763) (Coleoptera: Curculionidae) populations collected from Şanlıurfa, Adana and Kahramanmaraş province in Turkey were investigated by conducting the discriminating concentration tests and the concentration–mortality bioassays. Low (0.04 mg/L) and high (0.20 mg/L) discriminating concentration tests indicated that there was phosphine resistance of *S. oryzae*. The survival rates of field population of *S. oryzae* in Şanlıurfa, Adana and Kahramanmaraş ranged from 0 to 99%, 0 to 90% and 0 to 89% at the low discriminating concentration while it ranged from 0 to 83%, 0 to 46.5% and 0 to 28.5% at the high discriminating concentration respectively. Based on 50% mortality level (LC_{50}), the Şanlıurfa 4, Adana 7 and Kahramanmaraş 8 populations were 57.5, 28.9, and 16.3 times more resistant, respectively, than the susceptible population (Kahramanmaraş 4). In conclusion, this study revealed that phosphine resistance in *S. oryzae* is high in the examined areas of Turkey and some populations have levels of resistance that may pose challenges to the continued use of phosphine for their management.

Key words: Discriminating concentration, fumigation, phosphine resistant, rice weevil, *Sitophilus oryzae*, Turkey.

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INTRODUCTION

Total global grain production is around 2.978 billion tons in 2019 (FAO, 2022). Turkey's total grain production was nearly 31.9 million tons in 2021 (TÜİK, 2022), representing 1.29% of total global grain production. During storage, grains are attacked by a numerous pest, particularly insect species, which cause very serious quantitative losses and qualitative degradations. Among stored-grain insect pests, the rice weevil, *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae), is one of the most destructive primary pests attacking many common stored cereals including rice, wheat and maize, and has a worldwide distribution (Longstaff, 1981; Gomes, Rodriguez, Poneleit, Blake, & Smith, 1983; Grenier, Mbaiguinam, & Delobel, 1997). Stored and milled rice grains are prone to attack by *S. oryzae*, causing heavy economic losses, and both the adults and larvae feed on the carbohydrates in rice and wheat grains causing weight loss and contamination (Park, Lee, Choi, Park, & Ahn, 2003). Adams (1976) reported that *S. oryzae* causes substantial losses to stored grain amounting 18.30%. Phosphine (PH_3) is the primary fumigant used to protect the majority of grain and a variety of other stored commodities from insect pests (Chaudhry, 2000; Wang, Collins, & Gao, 2006). Attributes that contribute to wide-spread use of PH_3 are that it is relatively cheap, easy to apply, leaves no or minimal residues and can be used in many storage types and commodities (Nayak & Collins, 2008).

The lack of ideal airtight conditions for fumigation in leaky structures increases the frequency of control failures and thus increases the frequency of PH_3 fumigation (Pacheco, Sartori, & Taylor, 1990; Chaudhry, 2000; Benhalima, Chaudhry, Mills, & Price, 2004; Lorini, Collins, Daglish, Nayak, & Pavic, 2007). Phosphine fumigation is a long establishedlong-established effective method to control stored product insects, but its continuous and discriminate use has resulted in the evolution of resistant populations (Chaudhry, 2000; Collins, Daglish, Pavic, & Kopittke, 2005; Lorini et al, 2007; Pimentel, Faroni, Totola, & Guedes, 2007). Repeated applications of PH_3 in poorly sealed storage unit have been considered as the cause of the development of strong resistance (Friendship, Halliday, & Harris, 1986; Zeng, 1999). The resistance of stored-grain insect pests to PH_3 was first reported following a worldwide survey carried out by the Food and Agriculture Organization of the United Nations in 1972-73 (Champ & Dyte, 1976). In this study, PH_3 resistance was detected in 33 out of the 82 countries they surveyed involving 82 of the 849 populations tested. Strong PH_3 resistance in all key species of stored grains has been reported in a number of countries (Lorini et al, 2007; Pimentel, Faroni, Silva, Batista, & Guedes, 2010; Opit, Philips, Aikins, & Hasan, 2012; Daglish, Nayak, & Pavic, 2014; Sağlam, Edde, & Phillips, 2015).

Resistance is a threat to effective management of *S. oryzae*, with its PH_3 resistance reported from many countries around the world (Champ & Dyte, 1976; Price & Mills, 1988; Herron, 1990; White & Lambkin, 1990; Rajendran & Narasimha, 1994; Benhalima et al, 2004). Strong resistance to PH_3 was first recorded in *S. oryzae* in China in a 1995-1997 survey (Zeng, 1999). In this survey, a resistance level 337 times that of a susceptible strain was detected. The resistance level of *S. oryzae* in

India was reported in 1998 to have increased to 425 times that of a susceptible strain (Rajendran, 1999). Weakly resistant *S. oryzae* was found at a high frequency in most regions of Australia, with strong resistance occurring sporadically in field-collected strains (Emery, Collins & Wallbank, 2003). To date, there are few studies on the status of PH_3 resistance in stored grain insect pests in Turkey, Koçak, Schlipalius, Kaur, Thuck, Ebert, Collins, & Yilmaz, 2015; Koçak, Yilmaz, Alpkent, & Ertürk, 2018). Koçak et al (2015) reported that four Turkish population of *Tribolium castaneum* (Herbst, 1797) were tested through bioassays for determining PH_3 resistance phenotypes and all population exhibited high level of PH_3 resistance. However, it is obvious that there is very limited information on the status and monitoring of PH_3 resistance in stored-grain insect pests in Turkey. The lack of scientific information on the status of PH_3 resistance in stored-grain insect pests from different geographic locations in Turkey justifies the study of long-term monitoring of PH_3 resistance in these insect pest populations in all regions of Turkey. The objective of the present study is to determine resistance status and levels of PH_3 resistance in *S. oryzae* adults collected from grain storage facilities in Şanlıurfa, Adana and Kahramanmaraş province of Turkey.

MATERIAL AND METHODS

Test insects and insect culture

Sitophilus oryzae adults collected from three provinces of Turkey were used in the bioassays and cultured in the laboratory on whole wheat at 26 ± 1ºC and 65 ± 5 % relative humidity (RH). Insect populations were taken in Şanlıurfa, Adana and Kahramanmaraş provinces, which are located in the Eastern Mediterranean and South-eastern regions. For Şanlıurfa, Adana and Kahramanmaraş province, 9, 10 and 10 *S. oryzae* populations were collected respectively. Wheat grains were sampled in bulk with a spiral grain probe from nine to ten different wheat storage units per province. Additionally cylindrical plastic probe traps (STORGARD WB Probe II trap, TREECE, USA) were also used to collect *S. oryzae* adults in bulk stored-grain. A total of 29 different *S. oryzae* populations were collected from Şanlıurfa, Adana and Kahramanmaraş provinces from different grain storages and cultured in the laboratory after species identification according to Boudreaux (1969). Soft wheat (*Triticum aestivum* L., cultivar. Elbistan Yazlığı) with 11 ± 0.5 % moisture content used in insect culture was kept at -20 °C for approximately one week before being used for disinfestation, since it may be contaminated with other storage pests. The insects from infested wheat sampled taken from the grain storages were separated by using a 2 mm metal sieve (Retsch Brand, Retsch GmbH, Germany). After adding 250 g of wheat into 1 l glass jars, unsexed adults were added and the mouths of the jars were covered with a muslin cloth. The culture jars were kept at 26 ± 1 ºC and 65 ± 5% RH for 1 week in a fully dark environment and the adults were allowed to lay eggs. After 1 week, the insects were removed and the jars returned to above conditions. After 40-45 days, a new generation of adults was obtained. In the bioassay tests, the F_2 generations of the insects from field populations were used to determine the frequency of PH_3 resistance.

Phosphine fumigation procedure and gas analysis

One hundred thirty ml fumigation glass vials with the insects and 5 gr wheat, which are closed with aluminum cap with septa by using the crimper, were used in biological tests. Phosphine gas was obtained from a 10 l steel gas cylinder (volume; 1% PH_3 + 99% N_2) supplied from Linde Industrial Gases Company (Germany). The latex rubber ball with the septum was used for collecting PH_3 gas from the steel as cylinder. Gas-tight syringes (Hamilton, Bonaduz, Switzerland) were used for collecting PH_3 gas from gas sampling ball and injection of PH_3 gas into the fumigation glass vials (9.45 cm in height x 1.25 cm in diameter) for dosing. Target concentrations of gas were achieved by adding the required amount of the 1% PH_3 to the vial, following removal of the same volume of air, which was determined to meet the target concentration based on vial volume. Analysis and quantification of PH_3 concentrations achieved in laboratory fumigation jars generally followed the methods reported earlier in similar work (Sekhon, Schilling, Phillips, Aikins, Hasan, Corzo & Mikel, 2010, Opit et al, 2012). The gas chromatography (Shimadzu GC-2010 model, Shimadzu Corporation, Japan) with Flame Photometric Detector (FPD) and Rt®-Q-BOND capillary column (30 m length * 0.32 mm inner diameter * 10 µm film thickness, RESTEK GC Columns, USA) was used to determine the concentrations of PH_3 gas exposed to *S. oryzae* adults in biological tests. The injection temperature was set to 250 ºC, the column temperature to 120 °C and the detector temperature to 250 ºC. Nitrogen (N_2) was used as the carrier gas. The amount of PH_3 gas injected into the device was 25 µl. The retention time was 1.56 minute. All analysis of PH_3 gas were preceded by establishing a quantitative standard curve using known PH_3 concentrations that could then be used to derive the concentration in a jar via a regression equation derived from the standard curve. The PH_3 concentration for any given fumigation vial represented the average concentration for that vial measured 30 min after adding the gas and the concentration measured at the end of the exposure period just before venting the vial. There was no reduction of phosphine concentrations at the start and at the end of the measurements.

Phosphine resistance tests

Discriminating concentration tests

Discriminating concentration tests were used by FAO Method No. 16 (FAO, 1975) with the following modifications reported by Daglish & Collins (1999) to determine whether *S. oryzae* field populations had detectable resistance and their frequency of resistance. Each of *S. oryzae* field populations was exposed to low and high discriminating concentrations of 0.04 mg / l (29 ppm) and 0.20 mg / l (1436 ppm) respectively for 20 hours at 25 ± 1 ºC and 60 ± 5% RH by method modified by Daglish & Collins (1999). The results of discriminating concentration test were evaluated and resented by using the in Table 1 classification. Forty *S. oryzae* adults (1-2 week old) were added to each glass vial with a small quantity of diet (5 g of wheat) and the lids of the glass vial were closed with a rubber septum capped with aluminum crimp caps. Target concentrations were applied to each jar using small amounts of 1% PH_3 followed by quantitative GC-FPD as described above. For each insect population, five

fumigation vials with 40 *S. oryzae* adults and food media were separately exposed to the low and high discriminating concentrations. Test insects were held under these concentrations in a growth chamber for 20 h at a controlled temperature of 25 ± 1ºC, and then all vials were removed and held in fresh air for 14 d at 25 ± 1ºC and 60 ± 5 %RH, after which they were assessed for mortality. The beetles were removed from the vials and counted as live, moribund, or dead. Moribund and dead adults were placed in a 9-cm Petri dish containing a piece of filter paper moistened with 0.5 ml of water. These adults were then re-evaluated after 24 h for recovery. Five small vials (200 adults) were held under air in a fumigation jar to serve as experimental control beetles for inclusion in the concentration-mortality analysis as those exposed to 0 ppm PH_3, and were held and evaluated in the same way as fumigated beetles.

Table 1. Interpretation of discriminating concentration test results.

Low concentration 0.04 mg/l*	High concentration 0.20 mg/l**	Resistance classification
No survivors	No survivors	Susceptible
Survivors	No survivors	Weak resistance
Survivors	Survivors	Strong resistance

*FAO (1975) **Modification of Daglish & Collins(1999)

Lethal concentration tests

In order to determine the levels of PH_3 resistance in *S. oryzae* adults collected from grain storage facilities in Şanlıurfa, Adana and Kahramanmaraş provinces, one population for each province, which had the highest survival rate and one of PH_3-susceptible population was used for concentration-mortality tests. For concentration-mortality tests, each selected population was exposed to at least 6 to 8 different concentrations of PH_3 ranging from 0.014 to 0.76 mg/l for 20 h at 25 **±** 1°C and 65±5 % RH. Mortality assessments were conducted 14 days after PH_3 fumigation. Bioassay procedures were similar to discriminating concentration tests. In the determination of the most susceptible *S. oryzae* population, three susceptible field populations of *S. oryzae* which had no survival in both low and high discriminating concentrations (Şanlıurfa 7, Adana 1 and Kahramanmaraş 4) were exposed to concentration-mortality tests. Each selected population was exposed to 6 to 8 different concentrations of PH_3 ranging from 0.005 to 0.025 mg/l for 20 h at 25 **±** 1 °C and 65 **±** 5 % RH. Mortality assessments were conducted 14 days after PH_3 fumigation. According to the results of probit analysis, Kahramanmaraş 4 field population which had the lowest LC_{99} value (0.022 mg/l) was considered a PH_3-susceptible population.

Data analysis

In all biological tests, numbers of dead and live insect obtained on the 14th day after the fumigations were counted and the survival rates were calculated. Analysis of variance (ANOVA) was used to analyze percentage survival data after arcsine transformation to normalize the data. Percentage survival for each discriminating concentration was also adjusted for natural survival in controls using Abbott formula

before analysis and was then analyzed using one-way analysis of variance (factor; insect population). Differences between the means were determined using the Tukey Multiple Range Test at the 5% significance level. In order to calculate the lethal concentration values (LC_{50} and LC_{99}) of the PH_3-resistant *S. oryzae* populations, the concentration-mortality data obtained were subjected to probit analysis using the POLO-PC (LeOra Software, 1994) program. The resistance factor (RF_{50}) for each PH_3-resistant population was obtained by dividing the LC_{50} value estimated for each insect population by the LC_{50} value of the PH_3 susceptible population.

RESULTS

Discriminating concentration tests showed that survival rates at both low and high discriminating concentrations varied significantly among field populations of *S. oryzae* in Adana province (Table 2). Survival data at the low and high discriminating concentrations indicated that there was clear evidence of resistance in nine out ten field populations of *S. oryzae* in Adana province. Survival rate (%) of field population of *S. oryzae* ranged from 0 to 98.5% at the low discriminating concentration while it ranged from 0 to 46.5% at the high discriminating concentration. Numerically, Adana 3 and Adana 6 populations of *S. oryzae* had the highest survival rate, with 98.5 and 46.5% at the low and high discriminating concentrations, respectively. No survivor in the Adana 1 population of *S. oryzae* was obtained at both the low and high discriminating concentrations, which had no detectable resistance, referred as the susceptible population. Two out of ten populations of *S. oryzae*, Adana 2 and Adana 3, had weak resistance, which had survivors ranging from 95.5 to 98.5% at the low discriminating concentration, but no survivors were detected at the high discriminating concentration. Seven out ten populations of *S. oryzae*, Adana 4, 5, 6, 7, 8, 9, and 10, had strong resistance, with survival ranging from 10 to 89% and 7.5 to 46.5% at both the low and high discriminating concentrations respectively.

The survival rates at both the low and high discriminating concentrations varied significantly among field populations of *S. oryzae* in Kahramanmaraş province (Table 3). Discriminating concentration tests showed that there was resistance in eight out of ten field populations of *S. oryzae* in Kahramanmaraş province. Survival rate (%) of the field population of *S. oryzae* ranged from 0 to 90% and 0 to 28.5% at the low and high discriminating concentrations respectively. The Kahramanmaraş 5 population of *S. oryzae* had the highest survival rate, with 90 % at the low discriminating concentration while Kahramanmaraş 10 population had the highest survival rate, with 28.5% at the high discriminating concentration. There were no survivors in Kahramanmaraş 2 and Kahramanmaraş 4 populations of *S. oryzae* at either low or high discriminating concentrations, which demonstrates no detectable resistance. One out ten populations of *S. oryzae*, Kahramanmaraş 3 had weak resistance, with 23% survival at the low discriminating concentration, but no survivors were detected at the high discriminating concentration. Seven out ten populations of *S. oryzae*, Kahramanmaraş 1, 5, 6, 7, 8, 9 and 10, had strong resistance, with survival ranging from 21 to 90% and 4.5 to 28.5% at both the low and high discriminating concentrations, respectively.

Table 2. Survival rates (%) and phosphine resistance classification of field populations of *Sitophilus oryza*e adults collected from Adana province after 20-h exposure to discriminating concentrations (0.04 and 0.20 mg/L) of phosphine.

Insect population	Survival rate (%) ± S.E		Phosphine resistance status
	0.04 mg/l PH_3	0.20 mg/l PH_3	
Adana 1	0 ± 0 E	0 ± 0 E	Susceptible
Adana 2	95.5 ± 0 AB	0 ±0 E	Weak resistance
Adana 3	98.5 ± 0 A	0 ± 0 E	Weak resistance
Adana 4	37 ± 4 D	18 ± 3.9 BCD	Strong resistance
Adana 5	10 ± 4.4 E	7.5 ± 1.5 DE	Strong resistance
Adana 6	89 ± 3.4 AB	46.5 ± 6.5 A	Strong resistance
Adana 7	85 ± 2.9 AB	34 ± 4.2 AB	Strong resistance
Adana 8	75.5 ± 3.4 BC	17.5 ± 1.7 CD	Strong resistance
Adana 9	41 ± 9.2 D	21.5 ± 5 BCD	Strong resistance
Adana 10	64 ± 4.7 C	28.5 ± 2.8 BC	Strong resistance
F and P* value	$F_{9,40}$=71.04 P<0.0001	$F_{9,40}$=21.83 P<0.0001	

*One-way ANOVA was applied to the data. Means within a column with the same upper-case letter are not significantly different (Tukey test at 5% level).

Table 3. Survival rates (%) and phosphine resistance classification of field populations of *Sitophilus oryzae* adults collected from Kahramanmaraş province after 20-h exposure to discriminating concentrations (0.04 and 0.20 mg/L) of phosphine.

Insect population	Survival rate (%) ± S.E		Phosphine resistance status
	0.04 mg/l PH_3	0.20 mg/l PH_3	
Kahramanmaraş 1	12.5 ± 3.7 DE	4.5 ± 2.4 CD	Strong resistance
Kahramanmaraş 2	0 ± 0 E	0 ± 0 D	Susceptible
Kahramanmaraş 3	23 ± 3.9 CD	0 ± 0 D	Weak resistance
Kahramanmaraş 4	0 ± 0 E	0 ± 0 D	Susceptible
Kahramanmaraş 5	90 ± 2.8 A	20.5 ± 2.1 AB	Strong resistance
Kahramanmaraş 6	43.5 ± 4.1 BC	12 ± 1.6 BC	Strong resistance
Kahramanmaraş 7	21 ± 7.2 D	5.5 ± 2. 4 CD	Strong resistance
Kahramanmaraş 8	49.5 ± 6.4 B	12 ± 1.2 BC	Strong resistance
Kahramanmaraş 9	24.5 ± 8.3 CD	7 ± 1.4 CD	Strong resistance
Kahramanmaraş 10	71 ± 4.3 A	28.5 ± 3.2 A	Strong resistance
F and P* value	$F_{9,40}$=46.41 P<0.0001	$F_{9,40}$=27.17 P<0.0001	

*One-way ANOVA was applied to the data. Means within a column with the same upper-case letter are not significantly different (Tukey test at 5% level).

The survival rates at both the low and high discriminating concentrations varied significantly among field populations of *S. oryzae* in Şanlıurfa province (Table 4). Discriminating concentration tests showed that there was resistance in eight out of nine field populations of *S.oryzae* in Şanlıurfa province. Survival rate of the field population of *S. oryzae* ranged from 0 to 99% and 0 to 83% at the low and high discriminating concentrations, respectively. Şanlıurfa 2 population of *S. oryzae* had the highest survival rate, with 99 % at the low discriminating concentration while Şanlıurfa 4 population had the highest survival rate, with 83% at the high discriminating concentration. No survivor in Şanlıurfa 7 populations of *S. oryzae* was obtained at both the low and high discriminating concentrations, which had no detectable resistance. Eight out of nine populations of *S. oryzae*, Şanlıurfa 1, 2, 3, 4, 5, 6, 8, and 9, had strong resistance, with survival ranging from 57.5 to 99% and 11.5 to 83% at both the low and high discriminating concentrations, respectively.

Table 4. Survival rates (%) and phosphine resistance classification of field populations of *Sitophilus oryzae* adults collected from Şanlıurfa province after 20-h exposure to discriminating concentrations (0.04 and 0.20 mg/L) of phosphine.

Insect population	Survival rate (%) ± S.E		Phosphine resistance status
	0.04 mg/l PH_3	0.20 mg/l PH_3	
Şanlıurfa 1	97.5 ± 0.7 A	23.5 ± 3.9 D	Strong resistance
Şanlıurfa 2	99 ± 0.6 A	22 ± 5.7 D	Strong resistance
Şanlıurfa 3	91.5 ± 2.5 AB	78.5 ± 4.2 AB	Strong resistance
Şanlıurfa 4	94.5 ± 2 A	83 ± 2.5 A	Strong resistance
Şanlıurfa 5	88.5 ± 3.2 AB	63.5 ± 5.6 B	Strong resistance
Şanlıurfa 6	57.5 ± 10 C	11.5 ± 2DE	Strong resistance
Şanlıurfa 7	0 ± 0 D	0 ±0 E	Susceptible
Şanlıurfa 8	93 ± 2.9 A	61.5 ± 4.4 B	Strong resistance
Şanlıurfa 9	73 ± 2.5 BC	42.5 ± 2.6 C	Strong resistance
F and P* value	$F_{8,36}$=63.66 P<0.0001	$F_{8,36}$=59.93 P<0.0001	

*One-way ANOVA was applied to the data. Means within a column with the same upper-case letter are not significantly different (Tukey test at 5% level).

As concentrations of PH_3 discriminating concentration tests also indicated that the frequencies of PH_3 resistance in *S. oryzae* populations varied according to sampling locations (Fig. 1). Six and seven populations of *S. oryzae* in Şanlıurfa and Adana had high survival ranging from 61 to 100% at the low discriminating concentration respectively, whereas only one population in Kahramanmaraş had high survival. In the case of the high discriminating concentration, only Şanlıurfa had five populations with high survival while Adana and Kahramanmaraş had no populations with high survival. These findings indicated that the high resistance frequencies in *S. oryzae* populations were more common in Şanlıurfa than those in Adana and Kahramanmaraş while Kahramanmaraş had the lowest number of *S. oryzae* populations with high resistance frequency.

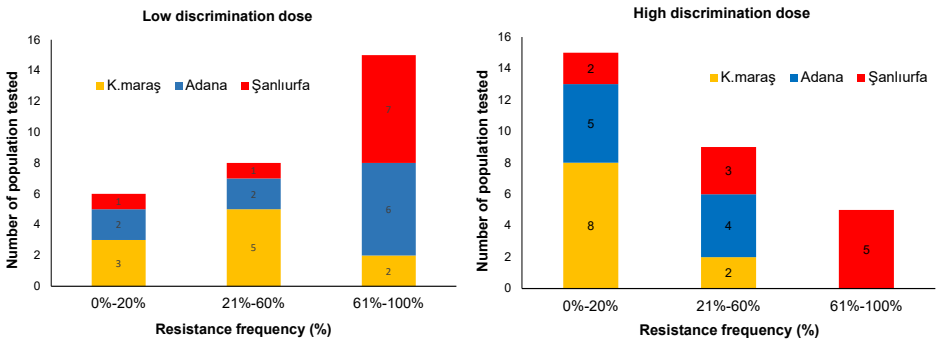

Fig. 1. Resistance frequency distribution for field populations of *Sitophilus oryzae* collected from Şanlıurfa, Adana and Kahramanmaraş province after 20-h exposure to (a) low (0.04 mg/L) and (b) high (0.20 mg/L) discriminating concentration of phosphine.

As concentrations of PH_3 required to kill 50 and 99% of the susceptible population (Kahramanmaraş 4) were compared with Adana 7, Şanlıurfa 4, Kahramanmaraş 8 population of *S. oryzae*, 95 % CLs of the susceptible population did not overlap those of Adana 7, Şanlıurfa 4, Kahramanmaraş 8 population, so the LC_{50} and LC_{99} values in each case were different. Therefore, in relation to LC_{50}, the Adana 7, Şanlıurfa 4, and Kahramanmaraş 8 populations were 28.9, 57.5, and 16.3 times more resistant than the susceptible population (Kahramanmaraş 4) respectively. Similarly, 95 % CLs of Şanlıurfa 4 population did not overlap those of Adana 7 and Kahramanmaraş 8 population, so the LC_{50} and LC_{99} values in each case were different. Based on LC_{50} values, Şanlıurfa 4 population was 2 and 3.5 times more resistant than Adana 7 and Kahramanmaraş 8 populations respectively Table 5. Finally, 95 % CLs of Adana 7 population did not overlap those of Maraş 8 population, so the LC_{50} and LC_{99} values in each case were different. Based on LC_{50} values, Adana 7 population was 1.77 times more resistant than Kahramanmaraş 8 population. It is important to note that the significant differences found in all comparisons existed despite the fact that heterogeneity values in all cases were >1, which implies the differences between the populations compared were quite pronounced.

Table 5. Probit analysis data of mortality for phosphine-resistant and susceptible field populations of *Sitophilus oryzae* resulting from 20-h laboratory fumigations at 25±1 °C and 65±5 % relative humidity.

Insect population	n[a]	Slope±SE	LC_{50} (mg/l) (95 % CI)[b]	LC_{99} (mg/l) (95 % CI)	χ^{2c}	(df)[d]	H[e]	RR[f]
Adana 7	1200	6.57±0.58	0.23 (0.20-0.25)	0.52 (0.47-0.63)	54.97	(28)	1.96	28.9
Şanlıurfa 4	1000	12.92±0.9	0.46 (0.45-0.48)	0.70 (0.66-0.77)	39.08	(23)	1.69	57.5
Kahramanmaraş 8	600	3.45±0.3	0.13 (0.10-0.15)	0.64 (0.46-1.03)	37.86	(18)	2.1	16.3
Kahramanmaraş 4*	1000	4.86±0.3	0.008 (0.007-0.009)	0.022 (0.026-0.033)	50.53	(23)	2.1	-

[a] Number of insects subjected to phosphine bioassay, excluding control, [b] Numbers in brackets give the 95% confidence intervals, [c] Chi square value, [d] Degree of freedom, [e] Heterogeneity value, [f] Resistance Ratio (RR) = Resistance Ratio (LC_{50} of resistant /LC_{50} of susceptible strain). * Susceptible field population.

CONCLUSION AND DISCUSSION

This study of PH_3 resistance in *S. oryzae* involved more extensive sampling than previous work in collecting field insects to assess the frequency and levels of resistance in some geographical locations in Turkey (Koçak et al, 2018). There are more populations with higher frequencies of resistance in our study compared with a similar study carried out in Turkey (Koçak et al, 2018). Low and high discriminating concentration tests indicated that there was clear evidence of PH_3 resistance in nine and eight out of ten field populations of *S. oryzae* (90 and 80%) in Adana and Kahramanmaraş respectively and eight out of nine populations (88.9%) in Şanlıurfa, whereas Koçak et al, (2018) found that only eleven out of 28 populations (39.3 %) of *S. oryzae* sampling from 14 provinces in Turkey had detectable resistance to PH_3. Similar to our study, Tingiş, Işikber, Sağlam, Bozkurt & Doğanay, (2018) reported that 89.9 and 83.3 % populations of tested *S. oryzae* populations collected from Mersin and Konya provinces respectively were resistant to PH_3, which reveals high prevalence of PH_3 resistance in the insect sampling locations of both provinces. In the present study, survival rates within field populations of *S. oryzae* in Adana, Kahramanmaraş and Şanlıurfa ranged from 0 to 98.5%, 0 to 90% and 0 to 99% at the low discriminating concentration while it ranged from 0 to 46.5%, 0 to 28.5% and 0 to 83% at the high discriminating concentration respectively. In the Tingiş et al, (2018) study, the ranges of these frequencies were 0-97.5% and 0-90% at the low and high discriminating concentrations for Mersin province, while they were 0-91.5% and 0-63% for Konya province respectively. These findings indicate that the high frequencies of resistance in *S. oryzae* populations are widespread in the grain storages sampled in Turkey, although the frequencies of PH_3 resistance varied according to the locations. Similarly, the resistance to PH_3 in *S oryzae* populations in many countries is increasing in frequency and strength. Although Champ & Dyte (1976) reported no resistance in Australian samples in 1976, less than two decades later resistance was detected in 35% of samples from the state of Queensland (White & Lambkin, 1990), 18% of samples from New South Wales (Herron, 1990) and 9% of samples from Western Australia (Emery, 1994). In India, the percentage of resistant strains increased from 30% during the early 1970s 10 to 72% in the 1990s (Rajendran, 1999).

PH_3 resistance was detected in 33 out of the 82 countries they surveyed involving 82 of the 849 populations tested (Champ & Dyte, 1976). At the time of that survey, the most resistant populations had 12 times more resistance than the susceptible ones. Reported resistance factors for unselected, or partially selected, field population of *S. oryzae* have increased from a maximum of 2.5× in the early 1970s (Champ & Dyte, 1976) to 52× in Australia (Nguyen, Collins & Ebert, 2015), 337× in China (Zeng, 1999) and 425× in India (Rajendran, 1999). And now, the present study shows that high levels of resistance occur in Turkish populations of *S. oryzae* as well. Based on 50% mortality level (LC_{50}), the Adana 7, Şanlıurfa 4, and Kahramanmaraş 8 populations in Turkey were 28.9, 57.5, and 16.3 times more resistant than the susceptible population (Kahramanmaraş 4) respectively. Tingiş et al (2018) and Koçak et al (2018) reported also a higher level of PH_3 resistance in different provinces in Turkey. In Tingiş et al (2018) study, based on the resistance factors (RF) calculated by LC_{50} values, *S. oryzae*

populations from Mersin and Konya province in Turkey were 102- to 104-fold and 38- to 81-fold more resistant to PH_3 compared with susceptible ones, respectively. Koçak et al (2018) also reported that based on LC_{50} values, *S. oryzae* populations from different provinces in Turkey were 3- to 200-fold more resistant to PH_3 compared with susceptible ones. These findings indicate that there are great variations in levels of PH_3 resistance in *S. oryzae* populations sampled from different provinces in Turkey.

In the present study, the frequencies of PH_3 resistance in *S. oryzae* populations varied according to sampling locations. The high resistance frequencies in *S. oryzae* populations were more common in Şanlıurfa than in those from Adana and Kahramanmaraş while Kahramanmaraş had the lowest number of *S. oryzae* populations with high resistance frequency. Şanlıurfa is known to have the highest production of wheat among the sampling locations (TUİK, 2020). Information from previous studies shows that *S. oryzae* is one of the most common stored-grain insect species in Turkey, and PH_3 is likely the only fumigant used in the control of stored grain insects in these provinces (Tingiş et al, 2018; Koçak et al, 2018). The high use of PH_3 can serve as an increased selection pressure for the resistance alleles. The adults of *S. oryzae* sampled from Kahramanmaraş had a lower resistance frequency, which could be due to very low frequencies of resistant genes under an environment of regular effective PH_3 use, or from minimal selection pressure of resistant genes due to low use of PH_3 in that province.

The gas concentration and exposure time (C × t product), in addition to temperature and RH, when fumigating with PH_3, plays a crucial role in its effectiveness for the most tolerant life stages of a pest species (Hagstrum et al, 2012). The frequency and level of PH_3 resistance in *S. oryzae* detected in the present study are based on experiments with a 20-h PH_3 exposure period at 25°C using adult beetles, which is the standard FAO method allowing us to discriminate between resistant to susceptible beetles in a simple way. Furthermore, the dose-mortality studies followed for a 20-h exposure time at 25°C to determine weak- and strong-resistant phenotypes based on PH_3 concentration alone. In the present study dose-mortality experiments clearly determined the weak- and strong-resistant phenotypes, but the calculated estimated concentrations required for 99% mortality should not be considered for practical fumigations to control infestations in the field, for which 20-h exposure period would be impractical and never recommended (Hagstrum et al, 2012).

The high level of PH_3 resistance detected among *S. oryzae* populations may be due to the previous selection pressure caused by inadequate fumigations in the storage facilities, from where the insect samples were collected. Other factors for differences in PH_3 resistance observed in the present study could be: fumigation in unsealed silos, lack of monitoring of PH_3 concentration during fumigation, and extensive use of only one fumigant (Benhalima et al, 2004; Wang et al, 2006; Lorini et al, 2007), favorable weather conditions for the insect pest (Collins et al, 2005; Lorini et al, 2007) and little or no insect management practiced in farms and others storage facilities (Pacheco et al, 1990; Lorini et al, 2007). In addition, the movement of insects as a result of the commodity trade is a likely contributor for the spread of PH_3 resistance in stored-product insects. As a result, lots of grains are fumigated frequently, and since the insect populations were

not successfully controlled, very high concentrations of PH_3 were used, increasing the likelihood selection for resistance (Collins et al, 2005; Lorini et al, 2007).

Phosphine gas is an important tool for the management of stored grain pests, and the occurrence of PH_3 resistance in pest populations presents challenges to the continued effective use of this fumigant. In the present study, the findings that PH_3 resistance was detected in nearly all populations of *S. oryzae* studied, with high frequencies of resistance in those populations, and the finding that many of these populations contained insects with the strong-resistant phenotype, point to the challenge pest managers are facing with PH_3 resistance. The levels of resistance in Turkey call for the use of an effective resistance monitoring program that contributes to fumigation operators to make decisions whether or not to use PH_3 in a given situation. Further work is required to continue monitoring for PH_3 resistance in Turkey, to determine strategies to manage resistance to PH_3 and to develop or use alternative fumigants such as sulfuryl fluoride, where PH_3 resistance has developed to very high levels (Opit, Thoms, Phillips & Payton, 2016) and also inert dusts, such as diatomoceus earth can be alternative for resistant strains (Korunic, 1998; Conceição, Faroni, Sousa, Pimentel, & Freitas, 2012; Sakka & Athanassiou, 2021).

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Docking-Based Virtual Screening Ascertaining β-Sitosterol-Induced Alterations in the *Helicoverpa armigera* Hübner Gut Enzymes

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ABSTRACT

Present investigation attempts to study binding of β-sitosterol with *Helicoverpa armigera* midgut enzymes; alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and alkaline phosphatase (ALP); through docking-based virtual screening. Extraction of the protein sequence of the enzymes revealed a respective linear chain of 535, 522 and 430 amino acids for ALP, ALT and AST. The binding energy for ALT-ligand complex was lowest as compared to the AST and ALP-docked complexes. The ALT-docked complex had ligand efficiency of (-) 0.32 with an inhibition constant of 104.01, more hydrogen bonds and hydrophobic interactions leading to a more stable complex. However, unfavored bumps in AST and ALP complexes may have led to comparatively unstable complexes. The dietary β-sitosterol exhibits differential binding with midgut enzymes of *H. armigera* larvae. The strong binding of β-sitosterol with ALT indicates the highest inhibition of ALT activity due to the activity-stability trade-off. The enzymes, AST and ALP exhibited relatively higher activity as a resultant of lesser stabilization of the β-sitosterol-enzyme complex. *In silico* studies have indicated that β-sitosterol can be used an effective control agent against *H. armigera.*

Keywords: Helicoverpa armigera, β-sitosterol, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), docking-based virtual screening.

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INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a serious threat to the global agricultural economy causing extensive annual crop damage worldwide (Craig & Jorge, 2013). Application of chemical-based interventions has led to the everlasting occurrence of insecticide residues in the foodstuff and drinking water, development of pest resistance against multiple pesticides and severe damage to the cultivation and beneficial organisms (Gill & Garg, 2014). All these issues have shifted the focus towards the development of phytochemical-based green chemistries and identification of the biochemical targets implicated in pest control and resistance management (Mouden, Klinkhamer, & Choi, 2017).

Bio-pesticides are considered safer and effective options owing to their non-detrimental nature, pest-specificity and biodegradability. Therefore, isolation, identification and characterization of bioactive phytocomponents from diverse flora inducing toxic, repellency/attraction and growth regulatory effects in insects is necessitated (Hikal Baeshen & Said-Al Ahl, 2017). *Thevetia neriifolia*, the yellow oleander plant, is an evergreen tropical shrub/small tree with antifungal, antibacterial properties and growth inhibitory potential against lepidopteran pest (Singh, Agarwal, Mishra, Mubbin, & Shukla, 2012; Mishra et al, 2015a, b; 2018; 2019). Our investigations on the effects of β-sitosterol on the physiological fitness and midgut enzymes of *H. armigera* revealed developmental abnormalities in *H. armigera* induced by the dietary β-sitosterol, an active phytocomponent identified from *T. neriifolia* stem extract (0.1-10 ppm) (Mishra et al, 2020). The larval and pupal weight gain reduced, the adult emergence dropped to 82.05% to 57.89% in comparison to 95.02% emergence in controls and the midgut biochemistry also altered significantly diminishing activity of three midgut enzymes; alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and alkaline phosphatase (ALP) (Mishra et al, 2020).

The GC-MS (Gas Chromatography-Mass Spectrometry) analysis of the extract discovered a number of phytocomponents with β-sitosterol as one of the major bioactive constituents. Though, β-sitosterol is used by the phytophagous insects for their growth and reproduction, it has also been reported as a feeding attractant (Hamamura, Hayashiya, & Naito, 1961).

Thus, the present investigation studied the β-sitosterol binding activity with the target midgut enzymes; ALT, AST and ALP through Docking-based virtual screening. The ligand-docking method saves us from technical complications, associated long time and high cost of massive experimental testing of millions of compounds (Koh, 2003; Tuccinardi, 2009). It plays a significant role in designing new drugs effective against pests during which a chemical library of existing ligands is docked into the target binding site and scored with the aim to generate a sub-library rich in potential binders (Cavasotto & Orry, 2007; Loza-Mejía, Salazar, & Sánchez-Tejeda, 2018).

We propose that the current study would help us to understand the affinity of β-sitosterol with target proteins alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and alkaline phosphatase (ALP) of *H. armigera via*

construction of a ligand library and, identify potential structural templates for efficient use in *H. armigera* management.

MATERIALS AND METHODS

Selection of enzymes

The earlier work has shown the inhibitory effect of β-sitosterol on three midgut enzymes - alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and alkaline phosphatase (ALP) of *H. armigera* in the order of ALT > AST > ALP with percent inhibition of 59.17, 42.51 and 29.31, respectively (Mishra et al, 2020). Thus, these enzymes were selected for docking-based studies to ascertain the effects of β-sitosterol in *H. armigera*.

Ligand preparation

The 3D SDF structure of the ligand, β-sitosterol was retrieved from PubChem database and later imported in discovery studio visualizer as a pdb file (https://pubchem.ncbi.nlm.nih.gov).

Target preparation

The peptide sequence of all the three midgut enzymes of *H. armigera*, ALT, AST and ALP was used to investigate the structure and catalytic sites. The sequence was extracted from National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/protein). The FASTA protein sequence database was subsequently submitted for sequence alignment and homology modelling.

Sequence alignment and modelling

The comparative modelling involves construction of a protein model based on the alignment of a submitted sequence to the target sequence. The modelling of the 3D structure of the enzymes was performed by homology modelling program. The N-terminal sequence was submitted in SWISS-MODEL to build up the structure of enzymes (https://swissmodel.expasy.org/interactive). It helped in predicting the tertiary structure of protein based on the primary structure submitted (https://swissmodel.expasy.org). The best 3D protein structure was obtained according to the best suitable template structures using BLAST database for similarity (Altschu, 1997; Bienert et al, 2017). The 3D protein model was validated through Ramachandran plot (Ramachandran, Ramakrishnan, & Sasisekharan,1963) using PROCHECK Validation Software (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK) (Laskowski, MacArthur, Moss, & Thornton, 1993). The residues information retrieved ascertained the stereochemistry of protein and provided the information on torsion angles (*psi* [Ø] and *phi* [Ö]). The final protein structure obtained was stored in discovery studio visualizer as a pdb file.

Molecular docking studies

The ligand and the target structures obtained above were exported to Autodock 4.2; assignments of charges and ionization were based on the standard templates as

a part of the Autodock software. The docking studies were carried out based on the protocol described by Rizvi, Shakil, & Haneef (2013). Standard software procedure was used. Briefly, Ligand PDBQT file was prepared by setting number of active torsions and aromaticity criterion as 5 and 7.5, respectively. Similarly, target PDBQT files were constructed by adding hydrogen atoms and Kollman charges. The parameter file was prepared by using the X, Y, Z dimension as 60 x 60 x 60 grid while the genetic algorithms and docking parameters were set on the standard templates as a part of Autodock 4.2 software for docking parameters. LamarkianGA(4.2) was selected as the output.

After docking, the dock score was calculated as binding energy of ligand with the target to assess the affinity of the ligand for the three targets tested.

RESULTS

The protein sequence of the three midgut enzymes in *H. armigera* extracted from NCBI revealed a linear chain of 522 amino acids (accession number XP_021189399) for ALT enzyme (Protein 1) whereas, the respective sequence for AST (Protein 2) and ALP (Protein 3) consisted of 430 amino acids (accession number XP_021185960.1) and 535 amino acids (accession number XP_021194992.1).

The best suited 3D structures of the proteins generated using SWISS-MODEL software based on the query sequence submitted are presented in Fig. 1. Protein 1 was made of 42% α-helix, 12% β-strand and 44% coil (Fig. 1a). In comparison, Protein 2 comprised 41% α-helix, 13% β-strand and 44% coil whereas, Protein 3 contained 30% α-helix, 11% β-strand and 58% coil (Figs. 1b, c). Ramachandran plot for ALT showed the presence of 90.1% residues in the favoured region while 8.7% additional residues were allowed in the additional region (Fig. 2a, Table 1). On the other hand, Ramachandran plots for AST and ALP revealed respective 93.8% and 89.1% residues in the favoured region whereas, additional allowed regions were 5.6% for AST and 9.5% for ALP (Figs. 2b, c; Table 1). The binding pockets for the enzymes were visualized using Castp 3.0 online server and active amino acids were obtained.

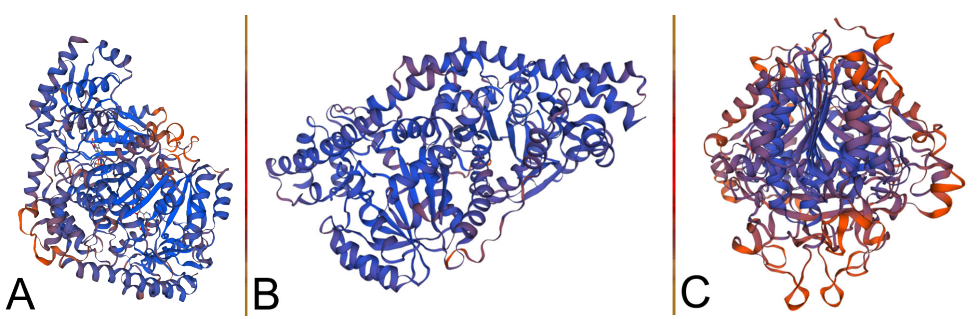

Fig 1. Best suited 3D modeled structure of (a) Alanine Aminotransaminase (ALT), (b) Aspartate Aminotransaminase (AST) and (c) Alkaline Phosphatase (ALP) enzymes using SWISS-MODEL Software.

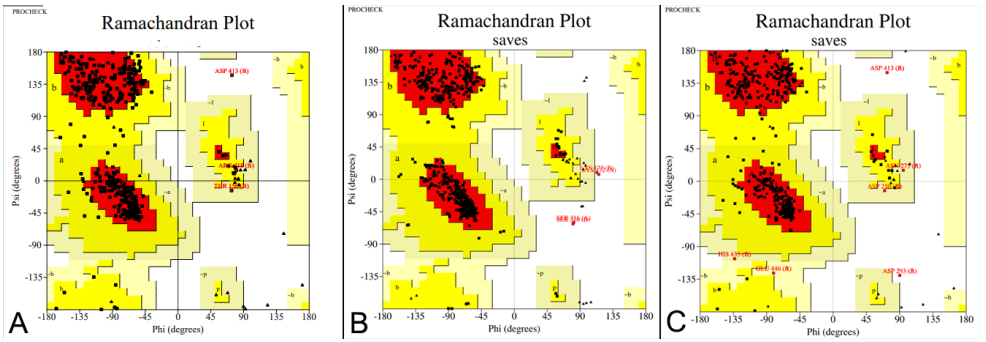

Fig 2. Ramachandran plots of (a) Alanine Aminotransaminase (ALT), (b) Aspartate Aminotransaminase (AST) and (c) Alkaline Phosphatase (ALP) enzymes obtained through Procheck validation server.

Table 1. Plot statistics based on Ramachandran Plot obtained using PROCHECK-Validation Server.

Residues	ALT	AST	ALP
Residues in most favoured regions [A,B,L]	736	653	754
Residues in additional allowed regions [a,b,l,p]	71	39	80
Residues in generously allowed regions [~a,~b,~l,~p]	7	0	8
Residues in disallowed regions	4	4	4
Number of non-glycine and non-proline residues	818	696	846
Number of end-residues (excluding Gly and Pro)	6	4	10
Number of glycine residues	76	60	78
Number of proline residues	50	40	36
Total number of residues	950	800	970

ALT = Alanine Aminotransaminase; AST = Aspartate Aminotransaminase; ALP Alkaline Phosphatase

The graphical analysis of the docked complexes for three tested proteins to view ligand-protein interactions using Autodock 4.2 software showed the best binding of the tested ligand in the binding pocket of ALT. The most important residues identified at the active binding pocket of ALT were Asp 134, Val 135, Gln 137, Arg 138, Asp 307 and Lys 311. The Asp 134, Val 135, ASP 307 was actively involved in formation of hydrogen bond with the ligand (Table 2, Fig. 3a). The binding energy obtained for ALT-ligand complex was (-) 9.53 kcal/mole whereas, the ligand efficiency was found to be (-) 0.32 with an inhibition constant of 104.01. On the other hand, the binding energies for AST (160.24 kcal/mole) and ALP (285.55 kcal/mole) complexes were quite higher. The docked complex for AST showed presence of 4 unfavored bumps (LEU 131, GLY 135, MET 314, TYR 315) whereas, ALP produced 7 unfavored bumps (SER 74, PRO 76, HIS 393, THR 396, ASP 470, ASP 471, GLY 468) produced due to amino acid group interactions (Figs. 3b, c). On the other side, presence of such bumps was not visualized in ALT complex.

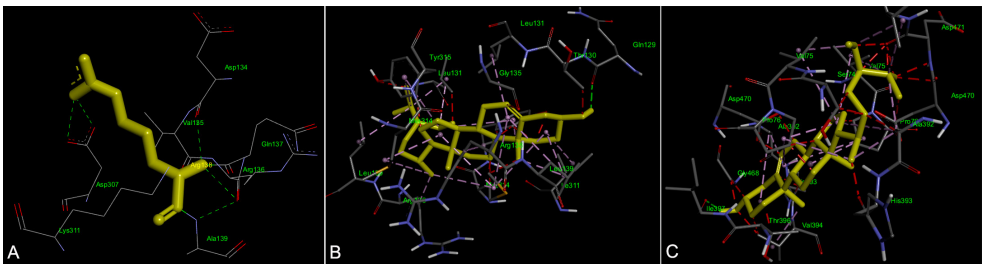

Fig 3. Ligand-protein complex of (a) Alanine Aminotransaminase (ALT), (b) Aspartate Aminotransaminase (AST) and (c) Alkaline Phosphatase (ALP) enzymes showing the actively participating amino acids binding with β-sitosterol.

Table 2. Interaction table of docking of Alanine Aminotransaminase (ALT), Aspartate Aminotransaminase (AST) and Alkaline Phosphatase (ALP) enzymes with β-sitosterol

Enzyme	H bond	Hydrophobic interactions	Hydrophilic interactions	Covalent interactions	Alkyl bond
ALT	ASP 134, VAL 135, ASP 307	ILE 58, LEU 61, VAL 135, ALA 139, ALA 310 ALA 369, MET 373,	Lys 130	ALA 139, GLN 137	LYS 311
AST	GLN 129	VAL 128, ALA 136, LEU 290	ARG 138, THR 130	NIL	ALA 136, ARG 138, LEU 290, VAL 128
ALP	ILE 397	ILE 118. MET 395, VAL 394	ASN 398, HIS 467	NIL	VAL 75, ALA 392, VAL 472

H-bonds = hydrogen bonds; ALA = alanine; ASP= aspartic acid; Val=Valine; ILE = isoleucine; LEU = leucine; ALA= alanine; MET = methionine; LYS=lysine; GLN= glutamine; ARG = arginine; THR = threonine

DISCUSSION

Plant-based insecticides offer a great means for the management of insect pests in a natural way. The pool of secondary metabolites in plants has been found to have a defensive role attributing to the diverse activities, such as antifeedant, insecticidal, growth regulatory etc. (Adeyemi, 2010). The multifarious bioactive molecules present in the plants are being explored continuously by the researchers owing to their non-detrimental effects on non-target hosts and nature (Adeyemi, 2010). The notable adverse effects of the dietary stem extract of *T. neriifolia* on the growth parameters, development and midgut enzymes of *H. armigera* larvae has already been demonstrated by us (Mishra et al, 2015a, b; 2018; 2019). The GCMS analysis of *T. neriifolia* stem extract revealed the presence of a number of components with β-sitosterol as one of the important bioactive constituents. As per our knowledge and reports available, the efficacy and probable use of β-sitosterol has not been investigated against *H. armigera,* though reported against a few other insects. Díaz et al, (2014) isolated β-sitosterol as the most active fraction from *Allophylus edulis* inducing growth regulatory effects on *Myzus persicae* and *Epilachna paenulata*. The antifeedant study against four lepidopteran pests viz., *H. armigera* (Hub.), *Spodoptera litura* (Fab.) (Fab.) and *Leucinodes orbonalis* revealed presence of steroids in the most effective fraction of the leaf extract of *Melochia corchorifolia* (Pavunraj, Baskar, & Ignacimuthu, 2012).

The bio-efficacy of sitosterol isolated from *Anemone pavonine* has been shown against *Pheidole pallidula* ants by Varitimidis, Petrakis, Vagias, & Roussis (2006).

Our earlier investigations showed the potential of β-sitosterol in disrupting physiological process and adequate growth and development which was proposed to be due to the accumulated effects of β-sitosterol and diminished midgut enzymatic activities (Mishra et al, 2020). As an extension of these studies, present investigations attempted to discover the exact binding sites of the target midgut enzymes with β-sitosterol through docking based virtual screening. We propose that the affinities and efficacy of the β-sitosterol against target enzymes can help in designing efficacious compound against *H. armigera*. Thus, β-sitosterol was used for construction of a ligand library aiming to identify potential structural templates.

The docked complex in our study showed more energetically favoured interaction of ALT with the ligand (β-sitosterol) as compared to AST and ALP protein. The presence of three hydrogen bonds and seven hydrophobic contacts in ALT-docked complex contributed to the stabilization with much lower binding free energy. The hydrogen bonds helped in tight gripping of the protein and ligand complex (Panigrahi, 2008; Rocher & Marchand-Geneste, 2008) while the hydrophobic and ionic interactions stabilized the docked complexes (Patil et al, 2010). Though AST-ligand docked complex also showed presence of Hydrogen bonds along with hydrophobic interactions, the unfavourable bumps caused due to different amino acids interaction may have attributed to a higher binding energy leading to an unstable complex. Similarly, ALP-docked complex displayed highest unfavored interactions that may have resulted in a very high binding energy of the complex as compared to other docked complexes. It has been reported that enzymes lose activity due to activity-stability trade-offs as they achieve enhanced stability by losing conformational dynamics (Siddiqui, 2017). On the other hand, enzymes gain additional dynamics and activity at the cost of stabilizing interactions. Present results are supported by our previous results that dietary β-sitosterol caused highest suppression of ALT enzyme activity (> 50%) in *H. armigera* larvae in comparison to the AST and ALP (Mishra et al, 2020). This proposes that decreased activity of ALT was accompanied by a concomitant increased stability caused by interactions and *vice-versa* in remaining two enzymes.

The expression of targeted genes has been studied earlier by applying *in silico* approaches by many researchers against various insects (Singhal, Kumar, & Virdi, 2014; Wickramasinghe et al, 2017). Another class of sitosterol, γ-sitosterol isolated from the methanolic extracts of *Hyptis suaveolens* possessed high binding affinity against odorant binding protein (3N7H) of *Anopheles gambiae* and was found to be an important mosquito repellent compound (Gaddaguti et al, 2012). Using AutoDock vina, Ahmad & Alkali (2018) tested nine phytochemical ligands for binding affinity with peroxisome proliferator-activated receptor gamma (PPAR-γ) involved in Diabetes-2 and obtained the best binding of β-sitosterol. On the other hand, Ma et al, (2015) reported slightly higher binding energy of three ligands; cholesterol, β-sitosterol and palmitic acid to sterol carrier Protein-2 which is a non-specific lipid transfer protein involved in the absorption and transportation of steroid or lipids in *H. armigera*. Other than insects,

the effect of binding of β-sitosterol to different receptors in other organisms has also been studied. Indriyanti, Garmana, & Setiawan (2018) conducted *in silico* assays with bryophyllin A, β-sitosterol, and kaempferol-3-glucoside isolated from *Kalanchoe pinnata* and tested their binding affinity with glucocorticoid receptor in mice. They revealed β-sitosterol binded to the receptor at Arg 611 and Gln 570 with hydrogen bonds and with hydrophobic binds at Gln 738, Try 735, Phe 737, Leu 741, Thr 739.

Present study provides sufficient information to identify the differential binding of midgut enzyme proteins in *H. armigera* larvae with dietary β-sitosterol. The study concludes the efficiency of β-sitosterol as an effective control agent against *H. armigera* suppressing the activity of midgut enzymes with highest inhibition of ALT activity due to the activity-stability trade-off. Other two enzymes, AST and ALP exhibited relatively higher activity as a resultant of lesser stabilization of the β-sitosterol-enzyme complex. Further binding affinity simulation studies may provide more evidence regarding control efficacy of β-sitosterol against *H. armigera.*

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CONFLICT OF INTEREST DISCLOSURE

There is no conflict of interest.

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Wing Venation Abnormalities in the Solitary Wasp Family Crabronidae (Insecta: Hymenoptera)

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ABSTRACT

Insect wings are flexible structures composed of tubular veins and thin wing membranes. In many insect groups, wings contain distinct taxonomic characters which are easy to describe (e.g., the number and length of veins, the wing size, etc.). However, some insects may have abnormal specimens that have some veins or their parts missing, or to the contrary have additional veins on the wings. In this study, forewing abnormalities in 248 species of 53 solitary wasp genera belonging to the family Crabronidae (Hymenoptera) collected from Turkey were investigated for the first time. As a result, forewing abnormalities were detected in 37 species belonging to 18 genera from five subfamilies. In total, 20 cases of wing venation abnormalities, classified as: a) supernumerary veins, b) defective veins and c) supernumerary cells, were observed in 67 of 3244 specimens. The abnormalities were rather common in following three species *Psammaecius punctulatus* (Vander Linden, 1829) (n = 8, 11.94%), *Bembix bidentata* Vander Linden, 1829 (n = 6, 8.95%), and *Bembecinus tridens* (Fabricius, 1781) (n = 4, 7.46%). *Nysson interruptus* (Fabricius, 1798) and *Nysson maculosus* (Gmelin, 1790) are species with more than one abnormality on the same wing. Abnormalities were generally observed in males (n=50, 74.63%) rather than females (n=17, 25.37%). The mechanism of this phenomenon, which is thought to occur due to genetic, environmental or pathogenic reasons, has not yet been clarified in many insect groups, including Crabronidae.

Key words: abnormalities, Crabronidae, defective veins, Hymenoptera, supernumerary cells, supernumerary veins.

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INTRODUCTION

Crabronidae is one of the most diverse families in the order Hymenoptera which collectively includes over 200 genera, containing well over 9000 species (Pulawski, 2022). The family consists of solitary and predatory wasps. This family includes quite different groups according to the variety of forewing veins and cells. Members of some groups of subfamilies such as Pemphredoninae and Crabroninae have different submarginal cell numbers in the forewings due to a reduction in some veins. Also, in some genera, the second submarginal cell is petiolated.

As in other families of the order Hymenoptera, the wing has a very important taxonomic characters in family Crabronidae. The following wing characters are frequently used for classification and identification of subfamilies, genera and species belonging to this family: number of submarginal cells, number of discoidal and subdiscoidal cells, whether submarginal and discoidal cells are separate or fused, size of stigma, terminal structure of the marginal cell, whether the second submarginal cell is petiolated or not, the shape and size of submarginal cells and the number and termination of recurrent veins (Bohart & Menke, 1976; Bitsch & Leclercq, 1993). However, due to wing abnormalities, changes may occur in these taxonomic characters and may lead to the misidentification of taxa or systematic problems (Gülmez, 2019).

Abnormalities are defined as physical developmental defects or malformations, and the exact causes are not known. Although abnormalities appear very rare in nature, abnormal specimens have been described in almost all animal groups, including insects. Balazuc (1958) provided a comprehensive classification of abnormality phenomena in the order Hymenoptera. Following this study, abnormalities such as gynandromorphism, irregularities in segmentation of gaster and antennae, supernumerary ocelli, loss of ocelli, loss of compound eye, and wing vein abnormalities have been reported in that order (Gülmez, 2019).

Mostly honey bees, *Apis mellifera* Linnaeus, 1758, have been well studied in terms of wing venation abnormalities within the order Hymenoptera (Soose, 1954; Akahira & Sakagami, 1959; Bährman, 1963; Torres & Ramos, 2000; Schneider & Feitz, 2003; Penteado-Dias, Nunes & Shimbori, 2005; Tan, Fuchs & Engel, 2008; Węgrzynowicz, Gerula, Panasiuk & Bieńkowska, 2010; Mazeed, 2011; Porporato, Laurino, Balzola & Manino, 2014; Caomério, Perioto & Lara, 2015; Eligül, Koca & Kandemir, 2017). A few studies on wing abnormalities of some ant species of the same order have also been reported (Perfilieva, 2000). Scarpulla (2018) summarized the cases with atypical and variable submarginal cell numbers on specimens belonging to the families Colletidae, Andrenidae, Halictidae and Apidae. Recently, wing abnormalities related to the Sphecidae family, which is in the same superfamily as honey bees in Hymenoptera, have been reported by Gülmez (2019). There is no record of any wing abnormalities related to the Crabronidae family.

In this study, various wing abnormalities were recorded for 69 specimens representing 37 species in the solitary wasp family Crabronidae (Hymenoptera). Abnormalities detected in specimens belonging to different genera from the Crabronidae family are presented.

MATERIALS AND METHODS

In this study, 3244 specimens belonging to 248 species of Crabronidae family were examined for wing venation abnormalities. Specimens were collected from Erzincan, Giresun, Gümüşhane, and Sivas provinces of Turkey between 2015 and 2020 and are deposited in the Entomology Research Laboratory, Department of Biology, Tokat Gaziosmanpaşa University (Tokat, Turkey). Abnormal wings (right or left) were removed from the specimens, placed between glasses in a slide frame and then photographed with a Leica M205C stereomicroscope controlled by the Leica Application Suite 3 software. The distribution of abnormalities among species is given in Table 1. The general shapes of the different types of forewings in the Crabronidae family are given in Fig. 1 with the names of veins and cells according to Bohart & Menke (1976) except for the terms “1st abscissa” and “2nd abscissa” which are from Porporato et al. (2014). Photographs representing the types of wing abnormalities belonging to different genera are given in the Figs. 2 - 9. In the present study, Porporato et al. (2014) was followed for the classification and naming of abnormalities.

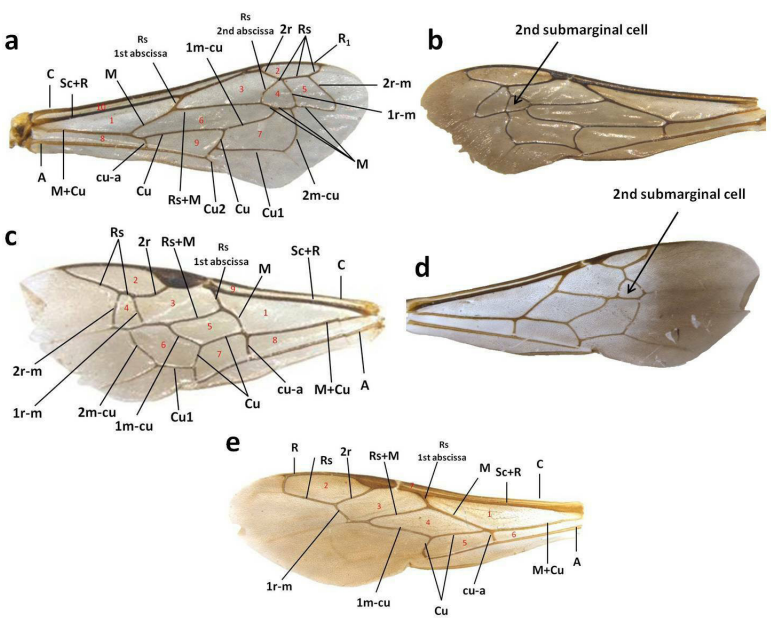

Fig. 1. Different types of forewings in the Crabronidae family in terms of cell number and veins. **a)** Right forewing of *Bembecinus tridens* with three submarginal cells representing cell and vein names. Cell names (1–10): 1. Medial, 2. Marginal, 3. Submarginal I, 4. Submarginal II, 5. Submarginal III, 6. Discoidal I, 7. Discoidal II, 8. Submedial, 9. Subdiscoidal, 10. Costal. Vein names: A. Anal, C. Costal, Cu. Cubital, M. Medial, R. Radial, Rs. Radial sector, Sc. Subcostal; **b)** Left forewing of *Brachystegus scalaris* with a petiolated second submarginal cell; **c)** Left forewing of *Diodontus minutus* with two submarginal cells representing names of cells and veins. Cell names (1-9): 1. Medial, 2. Marginal, 3. Submarginal I, 4. Submarginal II, 5. Discoidal I, 6. Discoidal II, 7. Subdiscoidal, 8. Submedial, 9. Costal. **d)** Right forewing of *Miscophus pretiosus* with a petiolated second submarginal cell; **e)** Left forewing of *Ectemnius crassicornis* with one submarginal cell representing names of cells and veins. Cell names (1-7): 1. Medial, 2. Marginal, 3. Submarginal, 4. Discoidal, 5. Subdiscoidal, 6. Submedial, 7. Costal.

Table 1. Distribution of abnormality types among species and their proportions.

Abnormality	Species	n	%a	%b
Supernumerary veins		**39**		
Spur protruding from 1r-m into the 2nd submarginal cell	*Astata kashmirensis* (1 ♂)	1	1.45	0.03
Spur protruding from 1m-cu into the 2nd discoidal cell	*Astata minor* (1 ♀) *Cerceris quinquefasciata* (1 ♂) *Nysson interruptus* (1 ♂)	3	4.35	0.09
Spur protruding from 2r into marginal cell	*Bembecinus tridens* (1 ♂) *Nysson pratensis* (1 ♂)	2	2.90	0.06
Spur protruding from Rs into marginal cell	*Bembecinus tridens* (1 ♀)	1	1.45	0.03
Spur protruding from 2m-cu	*Bembix bidentata* (1 ♀) *Bembix tarsata* (1 ♂) *Larra anathema* (1 ♀) *Tachysphex incertus* (1 ♂) *Tachysphex obscuripennis* (1 ♀) *Tachysphex panzeri* (1 ♂) *Tachysphex tessellatus* (1 ♂)	7	10.14	0.22
Spur protruding from 2m-cu into the 2nd discoidal cell	*Bembix bidentata* (1 ♂) *Nysson gerstackeri* (1 ♀) *Tachysphex incertus* (1 ♂)	3	4.35	0.09
Spur protruding from 2nd abscissa of Rs into 1st submarginal cell	*Bembecinus tridens* (1 ♂) *Gorytes quinquefasciatus* (1 ♂) *Prosopigastra bulgarica* (1 ♂) *Psammaecius punctulatus* (2♀♀, 5 ♂♂)	10	14.49	0.31
Spur protruding from RS+M into the 1st submarginal cell	*Nysson interruptus* (1 ♀) *Nysson variabilis* (1 ♂)	2	2.90	0.06
Spur protruding from 2nd abscissa of Rs into the 2nd submarginal cell	*Psammaecius punctulatus* (1 ♂)	1	1.45	0.03
Spur protruding from 2nd abscissa of Rs	*Ectemnius crassicornis* (1 ♀) *Nitela truncata* (1 ♀)	2	2.90	0.06
Spur protruding from 2r-m	*Astata kashmirensis* (1 ♂) *Astata minor* (1 ♂) *Cerceris stratiotes* (1 ♂) *Tachysphex pompiliformis* (1 ♂)	4	5.80	0.12
Spur protruding from 1m-cu into 1st discoidal cell	*Prosopigastra orientalis* (1 ♂)	1	1.45	0.03
Spur protruding from R1 into the marginal cell	*Nysson maculosus* (1 ♂) *Philanthus triangulum* (1 ♂)	2	2.90	0.06
Defective veins		**11**		
Incomplete 2r-m crossvein	*Nysson maculosus* (1 ♂)	1	1.45	0.03
Incomplete 1r-m crossvein	*Cerceris specularis* (1 ♂) *Miscophus pretiosus* (1 ♂) *Tachysphex brevipennis* (1 ♂) *Philanthus triangulum* (1 ♂) *Diodontus luperus* (2 ♂♂) *Diodontus minutus* (1 ♀) *Nysson interruptus* (1 ♂)	8	11.59	0.25
Incomplete 2m-cu crossvein	*Miscophus pretiosus* (1 ♂)	1	1.45	0.03
Incomplete 1m-cu crossvein	*Prosopigastra bulgarica* (1 ♂)	1	1.45	0.03

Table 1. Continued.

Abnormality	Species	n	%a	%b
Supernumerary cells		**19**		
Supernumerary submarginal cell	*Astata affinis* (1 ♀) *Bembecinus tridens* (1 ♂) *Bembix bidentata* (1 ♀, 3 ♂♂) *Brachystegus scalaris* (1 ♀) *Gorytes albidulus* (1 ♂) *Gorytes quinquecinctus* (1 ♀) *Nysson variabilis* (1 ♀) *Tachysphex fulvitarsis* (2 ♂♂) *Tachysphex graecus* (1 ♂) *Tachysphex obscuripennis* (1 ♂) *Tachysphex psammobius* (1 ♂)	15	21.74	0.46
Supernumerary discoidal cell	*Bembecinus tridens* (1 ♂) *Gorytes quinquefasciatus* (2 ♂♂)	3	4.35	0.09
Incomplete supernumerary discoidal cell	*Tachysphex brevipennis* (1 ♂)	1	1.45	0.03

%a Rate of total abnormal forewings

%b Rate of total examined material

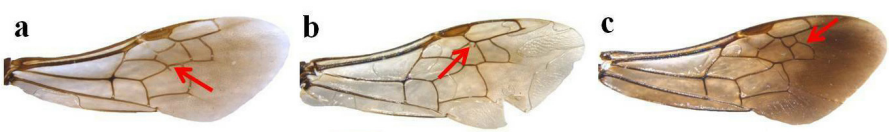

Fig. 2. Forewing abnormalities in the genus *Astata*. a) *A. minor* (♀), spur protruding from 1m-cu into 2nd discoidal cell; b) *A. kashmirensis* (♂), spur protruding from 1r-m into 2nd submarginal cell; c) *A. affinis* (♀), supernumerary submarginal cell.

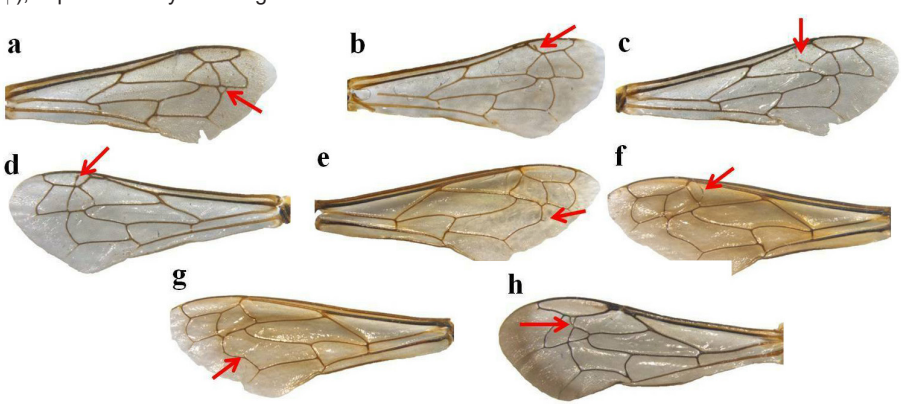

Fig. 3. Forewing abnormalities in the genus *Bembecinus*, *Bembix* and *Brachystegus.* a) *Bembecinus tridens* (♂), supernumerary discoidal cell; b) *Bembecinus tridens* (♂), spur protruding from 2r into marginal cell; c) *Bembecinus tridens* (♂), spur protruding from 2nd abscissa of Rs into the 1st submarginal cell; d) *Bembecinus tridens* (♀), spur protruding from Rs into marginal cell; e) *Bembix bidentata* (♀), spur protruding from 2m-cu; f) *Bembix bidentata* (♂), supernumerary submarginal cell; g) *Bembix bidentata* (♂), spur protruding from 2m-cu into the 2nd discoidal cell; h) *Brachystegus scalaris* (♀), supernumerary submarginal cell.

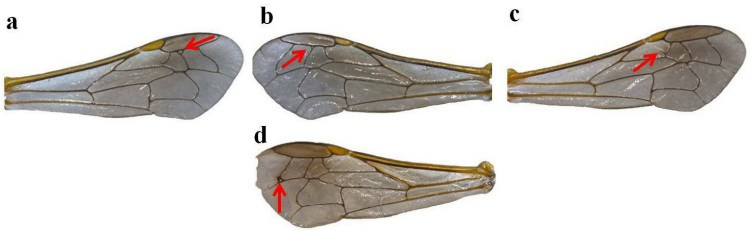

Fig. 4. Forewing abnormalities in the genus *Gorytes*. a) *Gorytes albidulus* (♂), supernumerary submarginal cell; b) *Gorytes quinquecinctus* (♀), supernumerary submarginal cell; c) *Gorytes quinquefasciatus* (♂), spur protruding from 2nd abscissa of RS into 1st submarginal cell; d) *Gorytes quinquefasciatus* (♂), supernumerary submarginal cell.

RESULTS

In this study, various forewing abnormalities were documented in 67 out of 3244 specimens (2.07%). Twenty different abnormalities have been identified which can be grouped under three main headings: a) supernumerary veins n = 39 (56.52%), b) defective veins n = 11 (15.94%) and c) supernumerary cells n = 19 (27.54%) (Table 1). The most common wing abnormalities are supernumerary veins which arise as spurs protruding from various veins, mostly from transverse ones. Reduction or deletion of some veins, defective vein abnormality, is the least common abnormality type which is found in transverse veins. Supernumerary cell formation is determined in submarginal and discoidal cells. The most common individual cases are: 1) supernumerary submarginal cell (n = 15, 21.74%), 2) spur protruding from 1st abscissa of Rs to 1st submarginal cell (n = 10, 14.49%) and 3) spur protruding from 2m-cu (n = 7, 10.14%). More than one abnormality in a same wing was observed in *Nysson interruptus* and *N. maculosus* (Figs. 5b and 5c).

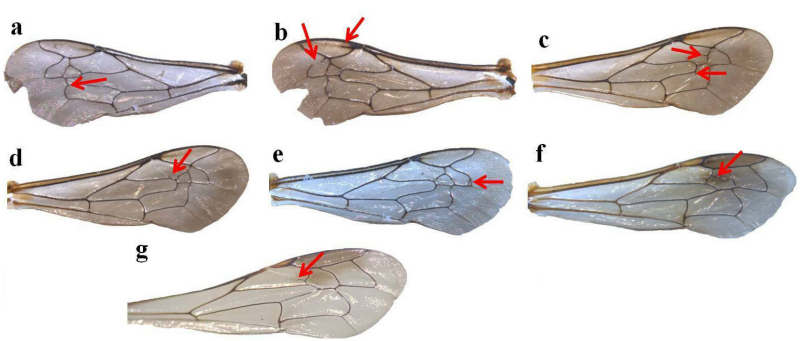

Fig. 5. Forewing abnormalities in the genus *Nysson*. a) *Nysson gerstaeckeri* (♀), spur protruding from 2m-cu into the 2nd discoidal cell; b) *N. maculosus* (♂), incomplete 2r-m crossvein and spur protruding from R1 into the marginal cell; c) *N. interruptus* (♂), spur protruding from 1m-cu into the 2nd discoidal cell and partially incomplete 1r-m crossvein; d) *N. interruptus* (♀), spur protruding from RS+M into the 1st submarginal cell; e) *N. variabilis* (♀), supernumerary submarginal cell; f) *Psammaecius punctulatus* (♂), spur protruding from 2nd abscissa of Rs into the 2nd submarginal cell; g) *Psammaecius punctulatus* (♀), spur protruding from 2nd abscissa of Rs into 1st submarginal cell.

When sexes are considered, their distribution are as follows: 50 males (74.63%) and 17 females (25.37%). The number of normal and abnormal species in the subfamilies and the percentage of species with abnormalities among all taxa studied are as follows: three species out of 12 in the subfamily Astatinae (1.21%), 13 species out of 57 in the subfamily Bembicinae (5.24%), 15 species out of 116 (6.04%) in the subfamily Crabroninae, two species out of 36 in the subfamily Pemphredoninae (0.80%) and four species out of 26 in the subfamily Philanthinae (1.61%).

Subfamily Astatinae Lepeletier de Saint-Fargeau, 1845

Genus *Astata* Latreille, 1797

In this study, totally 183 specimens (70 ♀♀ and 113 ♂♂) of nine species of the genus *Astata* were examined for their forewing abnormalities. Abnormalities were detected in five specimens belonging to *Astata affinis* Vander Linden, 1829 (1 ♀), *A. kashmirensis* Nurse, 1909 (2 ♂♂), and *A. minor* Kohl, 1885 (1 ♀, 1 ♂). Supernumerary cell (Fig. 2c) and supernumerary veins (Figs. 2a, 2b) were observed in these specimens.

Subfamily Bembicinae Latreille, 1802

Genus *Bembecinus* A. Costa, 1859

In this study, a total of 103 specimens (47 ♀♀ and 56 ♂♂) belonging to five species of the genus *Bembecinus* were examined for their forewing abnormalities. Abnormalities were detected only in *Bembecinus tridens* (Fabricius, 1781) (1♀, 4 ♂♂). Supernumerary cell (Fig. 3a) and supernumerary veins were observed in these specimens (Figs. 3b-d).

Genus *Bembix* Fabricius, 1775

In this study, a total of 82 specimens (42 ♀♀ and 40 ♂♂) belonging to six species of the genus *Bembix* were examined for their forewing abnormalities. Abnormalities were detected in *Bembix tarsata* Latreille, 1809 (1 ♂) and *B. bidentata* Vander Linden, 1829 (2 ♀♀, 4 ♂♂). Supernumerary cell (Fig. 3f) and supernumerary veins (Figs. 3e, 3g) were observed in these specimens.

Genus *Brachystegus* A. Costa, 1859

In this study, a total of 24 specimens (22 ♀♀ and 2 ♂♂) belonging to *Brachystegus incertus* Radoszkowski, 1877 and *B. scalaris* (Illiger, 1807) were examined for their forewing abnormalities. Supernumerary submarginal cell was detected as an abnormality in a female specimen belonging to *B. scalaris* (Fig. 3h).

Genus *Gorytes* Latreille, 1805

In this study, totally 89 specimens (35 ♀♀ and 54 ♂♂) of nine species of the genus *Gorytes* were examined for their forewing abnormalities. Abnormalities were detected in five specimens belonging to *Gorytes albidulus* Lepeletier de Saint-Fargeau, 1832 (1 ♂), *G. quinquecinctus* (Fabricius, 1793) (1 ♀), and *G. quinquefasciatus* (Panzer, 1798) (3 ♂♂). Supernumerary cell (Figs. 4a, 4b, 4d) and supernumerary veins (Fig. 4c) were observed in these specimens.

Genus *Nysson* Latreille, 1802

In this study, totally 105 specimens (58 ♀♀ and 47 ♂♂) of 12 species belonging to genus *Nysson* were examined for their forewing abnormalities. Abnormalities were detected in seven specimens belonging to *Nysson gerstaeckeri* Handlirsch, 1887 (1 ♀), *N. interruptus* (Fabricius, 1798) (1♀ 1 ♂), *N. maculosus* (Gmelin, 1790) (1 ♂), *N. pratensis* Mercet, 1909 (1 ♂), and *N. variabilis* Chevrier, 1867 (1♀ 1 ♂). Supernumerary cell (Fig. 5e), supernumerary veins (Figs. 5a-d) and defective vein (Fig. 5b) were observed in these specimens. Although both *Nysson interruptus* (♂) and *Nysson maculosus* (♂) are represented with one specimen, they are included in two different abnormality sections in Table 1 since they have more than one abnormality on the same wing.

Genus *Psammaecius* Lepeletier de Saint-Fargeau, 1832

In this study, a total of 37 specimens (17 ♀♀ and 20 ♂♂) belonging to *Psammaecius punctulatus* (Vander Linden, 1829) were examined for their forewing abnormalities. Supernumerary veins were detected in eight specimens (2 ♀♀ 6 ♂♂) (Figs. 5f, 5g).

Subfamily Crabroninae Latreille, 1802

Genus *Ectemnius* Dahlbom, 1845

In this study, a total of 96 specimens (40 ♀♀ and 56 ♂♂) of 13 species belonging to genus *Ectemnius* were examined for their forewing abnormalities. Only, a spur protruding from 2nd abscissa of Rs was detected in *Ectemnius crassicornis* (Spinola, 1808) (1 ♀) (Fig. 6a).

Genus *Larra* Fabricius, 1793

In this study, a total of 15 specimens (12 ♀♀ and 3 ♂♂) belonging to *Larra anathema* (Rossi, 1790) were examined for their forewing abnormalities. Only a supernumerary vein was detected in a female specimen (Fig. 6b).

Genus *Miscophus* Jurine, 1807

In this study, a total of 108 specimens (44 ♀♀ and 64 ♂♂) belonging to 11 species of genus *Miscophus* were examined for their forewing abnormalities. Only defective veins were detected in *Miscophus pretiosus* Kohl, 1884 (2 ♂♂) (Fig. 6c, 6d).

Genus *Nitela* Latreille, 1809

In this study, a total of 3 specimens (2 ♀♀ and 1 ♂) belonging to three species of genus *Nitela* were examined for their forewing abnormalities. Only supernumerary vein was detected in *Nitela truncata* Gayubo & Felton, 2000 (1 ♀) (Fig. 6e).

Genus *Prosopigastra* A. Costa, 1867

In this study, totally 118 specimens (37 ♀♀ and 81 ♂♂) of five species of the genus *Prosopigastra* were examined for their forewing abnormalities. Abnormalities were detected in three specimens belonging to *Prosopigastra bulgarica* Pulawski, 1958 (2 ♂) and *Prosopigastra orientalis* de Beaumont, 1947 (1 ♂). Supernumerary veins (Figs. 6h, 6g) and defective vein (Fig. 6f) were observed in these specimens.

Genus *Tachysphex* Kohl, 1883

In this study, totally 786 specimens (295 ♀♀ and 491 ♂♂) of 28 species of the genus *Tachysphex* were examined for their forewing abnormalities. Abnormalities were detected in 13 specimens belonging to *Tachysphex brevipennis* Mercet, 1909 (2 ♂♂), *T. fulvitarsis* (A. Costa, 1867) (2 ♂♂), *T. graecus* Kohl, 1883 (1 ♂), *T. incertus* (Radoszkowski, 1877) (2 ♂♂), *T. obscuripennis* (Schenck, 1857) (1♀ 1 ♂), *T. panzeri* (Vander Linden, 1829) (1 ♂), *T. pompiliformis* (Panzer, 1804) (1 ♂), *T. psammobius* (Kohl, 1880) (1 ♂), and *T. tessellatus* (Dahlbom, 1845) (1 ♂). Supernumerary cells (Figs. 7e-j), supernumerary veins (Figs. 7b-d) and defective vein (Fig. 7a) were observed in these specimens.

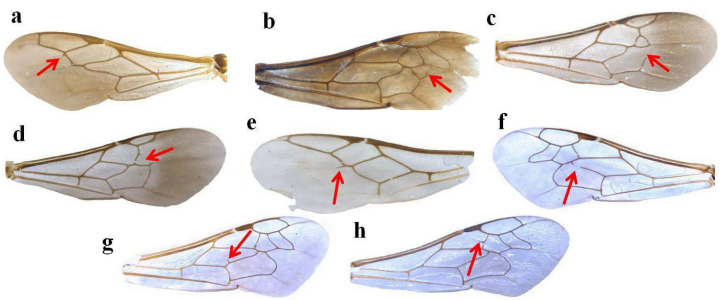

Fig. 6. Forewing abnormalities in the genera *Ectemnius, Larra, Miscophus, Nitela, and Prosopigastra.* a) *Ectemnius crassicornis* (♀), spur protruding from 1r-m; b) *Larra anathema* (♀), spur protruding from 2m-cu; c) *Miscophus pretiosus* (♂), incomplete 2m-cu crossvein; d) *Miscophus pretiosus* (♂), partially incomplete 1r-m crossvein; e) *Nitela truncata* (♀), spur protruding from submarginal cell; f) *Prosopigastra bulgarica* (♂), incomplete 1m-cu crossvein; g) *Prosopigastra orientalis* (♂), spur protruding from 1m-cu into 1st discoidal cell; h) *Prosopigastra bulgarica* (♂), spur protruding from 2nd abscissa of Rs into 1st submarginal cell.

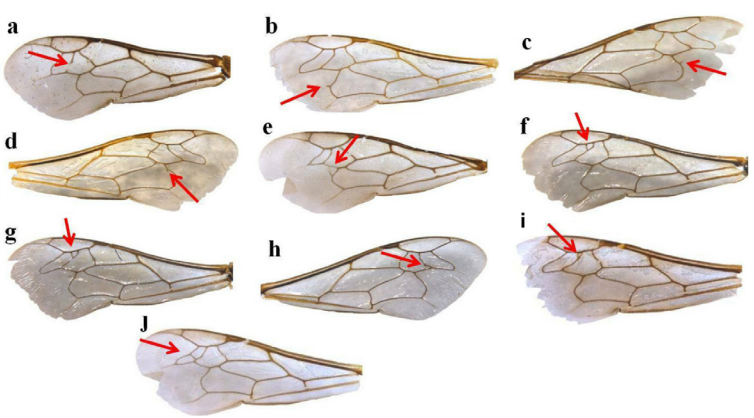

Fig. 7. Forewing abnormalities in the genus *Tachysphex*. a) *Tachysphex brevipennis* (♂), incomplete 1r-m crossvein; b) *Tachysphex incertus* (♂), spur protruding from 2m-cu; c) *Tachysphex obscuripennis* (♀), spur protruding from 2m-cu; d) *Tachysphex incertus* (♂), spur protruding from 2m-cu into the 2nd discoidal cell; e) *Tachysphex brevipennis* (♂), incomplete supernumerary discoidal cell; f) *Tachysphex fulvitarsis* (♂), supernumerary submarginal cell; g) *Tachysphex fulvitarsis* (♂), supernumerary submarginal cell; h) *Tachysphex graecus* (♂), supernumerary submarginal cell; i) *Tachysphex obscuripennis* (♂), supernumerary submarginal cell; j) *Tachysphex psammobius* (♂), supernumerary submarginal cell.

Subfamily Pemphredoninae Dahlbom, 1835

Genus *Diodontus* Curtis, 1834

In this study, a total of 87 specimens (45 ♀♀ and 42 ♂♂) belonging to five species of the genus *Diodontus* were examined for their forewing abnormalities. Abnormalities were detected in *Diodontus luperus* Shuckard, 1837 (2 ♂♂) and *Diodontus minutus* (Fabricius, 1793) (1 ♀). Defective veins were observed in these specimens (Figs. 8a, 8b).

Subfamily Philanthinae Latreille, 1802

Genus *Cerceris* Latreille, 1802

In this study, a total of 208 specimens (62 ♀♀ and 146 ♂♂) belonging to 21 species of the genus *Cerceris* were examined for their forewing abnormalities. Abnormalities were detected in *Cerceris quinquefasciata* (Rossi, 1792) (1 ♂), *C. specularis* A. Costa, 1867 (1 ♂) and *C. stratiotes* Schletterer, 1887 (1 ♂). Supernumerary veins were observed in these specimens (Figs. 9a, 9b).

Genus *Philanthus* Fabricius, 1790

In this study, a total of 79 specimens (32 ♀♀ and 47 ♂♂) belonging to four species of the genus *Philanthus* were examined for their forewing abnormalities. Abnormalities were detected in *Philanthus triangulum* (Fabricius, 1775) (2 ♂♂). Defective vein (Fig. 9d) and supernumerary vein (Fig. 9c) were observed in these specimens.

DISCUSSION

In this study, a large number of specimens belonging to the Crabronidae family were evaluated for the first time in terms of wing abnormalities. Forewing abnormalities were detected in 37 species of 18 genera out of 248 species belonging to 53 genera.

Abnormal forewing was detected in all of the studied subfamilies except the Dinetinae. The subfamily Crabroninae is the one with the highest number of abnormal species, followed by Bembicinae. While the most abnormal specimens were detected in the Bembicinae subfamily, the lowest number of abnormal species and specimens was detected in Pemphredoninae compared to other subfamilies. Although Crabroninae is twice as large as Bembicinae in terms of the number of examined specimens and species, the number of abnormal specimens is higher in the Bembicinae. The wing structures of the subfamilies and genera belonging to the Crabronidae differ considerably in terms of the number of veins and cells. While subfamilies and genera belonging to this family have one (Fig. 1e), two (Fig. 1c) and three submarginal cells (Fig. 1a); in some of these, the second submarginal cell is petiolated (Fig. 1b, 1d). The reduction in the number of veins and cells in the forewings of some subfamilies may have caused the abnormalities to be observed less frequently. However, it may not be an accurate assessment to evaluate abnormalities between subfamilies due to differences in vein and cell numbers.

The highest number of abnormal species was detected in *Tachysphex* with nine species, followed by *Nysson* with five species. *Tachysphex* is the most studied genus

with 28 species and 786 specimens, so this may have caused the overestimation of the number of abnormal species in this genus. *Psammaecius punctulatus*, *Bembix bidentata* and *Bembecinus tridens* were determined as the species with the most abnormal specimens (Table 1).

Supernumerary veins are found to be more common than other main abnormalities in this study; these results are consistent with the findings obtained by Porporato et al. (2014) and Gülmez (2019) in honey bees (*Apis mellifera*) and solitary wasps (Sphecidae), respectively. The spur protruding from 1m-cu to the 1st discoidal cell and the adventitious distal abscissa of the 2rs-m crossvein are the most frequently detected abnormalities in Gülmez (2019) and Porporato et al. (2014), respectively. However, unlike the results of those studies, the supernumerary submarginal cell is the most common abnormality in this study. It has been stated that 1r-m, 2r-m and 1m-cu veins show high variability and instability in the venation of the Aculeata wings (Akahira & Sakagami, 1959; Zimmermann, 1933; Alpatov, 1929; Porporato et al., 2014; Gülmez, 2019). In this study, 1r-m abnormalities among defective veins are common and it can be said that it is also unstable in the Crabronidae family. In both *Miscophus pretiosus* and *Nysson interruptus*, which have petiolated second submarginal cell, one edge of it is missing (Fig. 5c, 6d). In this respect, it is considered to be quite interesting among abnormal samples.

The number of submarginal cells in forewing of Crabronidae is a widely used feature to distinguish the genera. When the number is reduced to two, it is sometimes impossible to know whether the missing vein is the 2nd abscissa of Rs or 1r-m. In some previous studies on the Apoidea superfamily, it was estimated which crossvein was reduced by looking at the size of the submarginal cells. The first crossvein was considered to be missing if the first submarginal cell was longer than the second (Tofilski, 2011). In some cases, the sizes of both cells are close to each other. It has been suggested that the first crossvein is mostly missing in the Hylaeinae subfamily (Michener, 2000), while in the Panurginae subfamily the second crossvein is missing (Robertson, 1925). However, there is no information about which crossvein is missing in genera with two submarginal cells in the Crabronidae family. This situation was encountered in *Diodontus* species with two submarginal cells in which an abnormality was detected (Fig. 8a, 8b). The detected abnormality also occurred in the crossvein between the submarginal cells. In this study, it was assumed that the 2nd abscissa of Rs crossvein of the forewings was reduced in *Diodontus* forewings with two submarginal cells, since the first submarginal cell is longer than the second.

Although the exact causes of wing abnormalities are not known, there may be many different causes such as genetic origins, mutations or environmental factors. It has been suggested that abnormalities may be due to genetic reasons in some specimens from the Diptera, Lepidoptera and Coleoptera orders (Yokoyama, 1959; Barigozzi, 1963; Sokoloff, 1966). On the other hand, it is stated that various environmental effects such as physical, nutritional and chemical factors, infectious microorganisms or toxins can cause non-genetic abnormalities in wing formation by acting on the developmental stage of insects (Gülmez, 2019). It is not possible to reach a conclusion about the

effect of environmental conditions on wing abnormalities in Crabronidae, since the developmental stages of wasps in the nests cannot be followed. In this study, a case of parasitism, which is one of the environmental factors, was observed on the adult wasp with wing abnormality. A parasitic event, stylopization, produced by insects belonging to the order Strepsiptera, was detected in the abdomen of an abnormal specimen of *Bembecinus tridens*. It is not known exactly whether the parasite enters the body during the pre-adult period or in the adult period. If the parasite entered during the development period, this parasitic condition may have caused the forewing abnormality observed in this specimen.

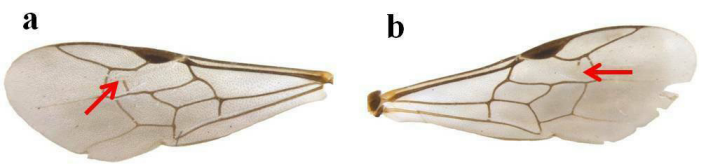

Fig. 8. Forewing abnormalities in the genus *Diodontus*. a) *Diodontus luperus* (♂), incomplete 1r-m crossvein; b) *Diodontus minutus* (♀), incomplete 1r-m crossvein.

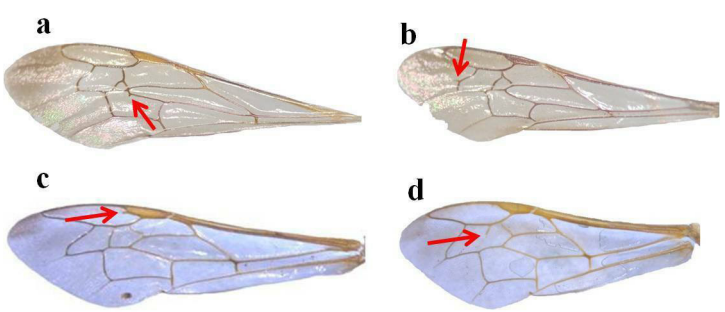

Fig. 9. Forewing abnormalities in the genera *Cerceris* and *Philanthus*. a) *Cerceris quinquefasciata* (♂), spur protruding from 1m-cu into 2nd discoidal cell; b) *Cerceris stratiotes* (♂), spur protruding from 2r-m; c) *Philanthus triangulum* (♂), spur protruding from R1 into the marginal cell; d) *Philanthus triangulum* (♂), incomplete 1r-m crossvein.

Especially in all specimens of *Psammaecius punctulatus*, the abnormalities were detected on the same veins in both wings. It has been stated that the causes of abnormalities in these species may be of either genetic or epigenetic origin (Gülmez, 2019). However, there are no studies which report the presence of genetic or epigenetic mechanisms leading to wing anomalies in solitary wasps.

The mechanism underlying wing vein formation in the vast majority of insects, including the order Hymenoptera, remains largely unexplored. The assessments regarding the causes of the wing abnormalities of the solitary wasps mentioned above generally remain at the level of predictions and assumptions. In order to find the causes of wing abnormalities, it is necessary to conduct studies on the molecular mechanisms of wing venation in solitary wasps.

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Some Biological Characteristics and Life Table of *Heliothis peltigera* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae) on Safflower

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ABSTRACT

Biology and life table of *Heliothis peltigera* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae), an important safflower pest, studied on two safflower varieties (Balcı and Dinçer) in the laboratory (25 ± 1 °C temperature, 65 ± 5% relative humidity and 16: 8 photoperiod). The longer pre-adult time of *H. peltigera* was found on Dinçer variety than Balcı variety, as 33.97±0.16 days, and 32.82±0.23 days, respectively. While the higher intrinsic rate of increase (*r*) (0.134±4.59 d^{-1}), and the finite rate of increase (*λ*) (1.144±0.005 d^{-1}) were estimated on Balcı variety, also shortest mean generation times were found on Balcı variety (37.49±0.34 days) than Dinçer variety (0.119±5.123 d^{-1}, 1.126±0.005 d^{-1}, and 39.25±0.40 days, respectively). According to these results, it was determined that Balcı variety was more suitable host plants than Dinçer variety for *H. peltigera*. These basic informations obtained in this study on *H. peltigera* will be useful for pest control and prefer to produce Dinçer variety that could be reducing of *H. peltigera*.

Key words: Bordered straw, biology, biological parameters, *Heliothinae*, pest management

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INTRODUCTION

The Bordered Straw (BS)*, Heliothis peltigera* Denis & Schiffermüller (Lepidoptera: Noctuidae) which is mainly distributed in the Paleosubtropic- Paleotropical regions (Matov, Zahiri, & Holloway, 2008), it is a well known migratory species in the Mediterranean Basin and Near and Middle East (Matov et al, 2008). It spread to new countries such as Canada and New Zealand with the effect of global warming and recently became a potentially important pest in Europe (Ragionieri et al, 2017). *Heliothis peltigera,* which is a polyphagous species is a pest of economically important plants such as tomato, cotton, corn, soybean, sunflower, bean and especially safflower besides some weeds and aromatic plants (Manjunath, Patel, & Yaoav, 1976). It has 3 generations a year in safflower fields in Iran and spends the winter under the soil as a pupa. Their larvae are fed in safflower leaves and capsules (Parvin, 1990).

Heliothis peltigera has been recorded on vegetable, corn and ornamental plants in warm regions where the Mediterranean climate in Turkey, in Adapazarı and Istanbul (Keyder, 1961); on cotton in the Aegean Region (İyriboz, 1971), on cotton, chickpeas and corn in Adana, Antalya, Hatay and İçel (Kornoşor & Düzgüneş, 1980), and on culture thyme in Turgutlu and Salihli (Tezcan, Okyar, & Beyaz, 2004). This pest was first reported by Damkacı (2013) on safflower in the Central Anatolia (Konya) region with a continental climate, and then from Niğde (Hüseyinoğlu & Atay, 2017). Eroğlu et al, (2019) collected 357 larvae *of H. peltigera* feeding on safflower in Adana to investigate the contents of NPV (Nucleopolyhedrovirus) and obtained a new isolate. *H. peltigera* has pests in the safflower fields in Ankara and has become a problem of safflower growers since 2014. The prevalence and incidence of *H. peltigera* in Ankara safflower areas were 96%, 2.5%, and 98.7%, 79.80% in 2018-2019, respectively (Ayten & Ülgentürk, 2020).

Studies on *H. peltigera* in Turkey and the world are determining its prevalence, host plants and natural enemies in a region. There is scarcely information about the biology of this species. The results presented by Ayten & Ülgentürk (2020) show that this species has a high spreading and significant pest potential. Having sufficient information about the population size, age distribution, growth rate, reproduction and survival rate of a pest is critical in control. Without this information, it is impossible to estimate the growth rate and damage of a pest population, the time and method of control and the number of these practices (Atlıhan, Özgökçe, & Hsin, 2018). Life tables are a fundamental tool used to study insect populations, resulting in crucial information for integrated pest management programs. For this reason, knowing the bio-ecology and life table of the pest is necessary to make an effective control against *H. peltigera*. In this study, the biological characteristics of *H. peltigera*, which is polyphagous and can be an important pest, are determined in laboratory conditions and a life table was created with the data obtained.

MATERIAL AND METHODS

Cultivation of safflower and *Heliothis peltigera*

Two varieties (Balcı and Dinçer) of safflower (*Carthamus tinctorius* L., Asteraceae), which are the hosts of *H. peltigera* (BS) and which are mostly grown by the producers, were obtained from Eskişehir Transitional Zone Agricultural Research Institute. While Balcı is a prickly variety and has an oil content of 31%, Dinçer, a prickless variety, has an oil content of 28.12% (Arslan & Çulhan, 2020). Safflower plants needed for feeding *H. peltigera* larvae were grown in Ankara Plant Protection Central Research Institute's garden. The stock culture was established from individuals obtained from eggs laid by *H. peltigera* adults collected from Ankara safflower fields.

Determination of the biology of *Heliothis peltigera* and creating a life table

The studies were conducted with leaves of safflower varieties, Balcı and Dinçer, at 25 ± 1 °C temperature, 65 ± 5% RH and 16: 8 (Light:Dark) photoperiods. Biology studies were started with 142 individuals (eggs) on Balcı and 146 individuals (eggs) on Dinçer when *H. peltigera* adults lay eggs. One egg was transferred to 9 cm diameter plastic Petri dishes containing blotter paper and fresh safflower leaves (separately for each variety of safflower). These Petri dishes were monitored daily, the eggs and larval stages were determined. The end of larval stages, they were transferred to plastic containers with sterilized soil at a height of 10-15 cm to follow the pupal stage and checked daily. Then, one adult female and one male were taken to a container that its top was covered with cheesecloth. Cotton soaked in 10% honey + 10% sugar water solution was placed in a container to feed to adults (Shorey & Hale, 1965). With the daily monitoring of adult individuals that its larvae feeding in two safflower varieties, the duration of life, pre-oviposition, oviposition and post-oviposition periods and fecundity of female individuals were determined. Observations were continued daily until the last individual died.

The pupal and adult sex ratio was determined by examining the genital segments of the pupae and adults grown in the laboratory using Leica M binocular microscope.

The raw life table data of *H. peltigera* reared on two safflower varieties were analyzed according to Chi & Liu (1985) and Chi (1988) using the computer program TWOSEX-MSChart (Chi, 2018; Wei et al, 2020). The variances and standard errors of population parameters were evaluated using the bootstrap technique (HesHesterberg, Moore, Monaghan, Clipson, & Epstein, 2005; Özgökçe, Chi, Atlıhan, & Kara, 2018) with 100,000 resampling to obtain stable estimates.

The mean development time of each stage of *H. peltigera*, adult female pre-oviposition period (*APOP*), total pre-oviposition period (*TPOP*), the total longevity of adults, gross reproductive rate (*GRR*), and mean number of eggs were calculated. The intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0) and mean generation time (T_0) were also determined using the computer program, TWOSEX-MS Chart (Chi, 2021). Based on life table data, population size of *H. peltigera*

was estimated using the computer program Timing-MSChart (Chi, 2019a) according to the method reported by Chi and Liu (1985) and Chi (1990). In this method, when a certain number of individuals are taken as the initial population of *H. peltigera,* the size that the population can reach at the end of the period to be determined can be estimated from the total number of individuals specific to age (*x*) and stage (*j)*.

RESULTS AND DISCUSSION

Development time and adult life

The egg development times of *H. peltigera* fed on Balcı (3.19±0.07) and Dinçer (3.23±0.07) safflower varieties were found to be statistically similar (P>0,05). A mean of 439.2 (282-1240) larvae emerged from 72% of the mean 610 eggs obtained on Balcı. A mean of 344 (149-1052) larvae emerged from 65% of the 531 eggs obtained on Dinçer. The difference between the number of larvae obtained depending on the variety was found to be statistically significant (P<0,05).

In our research, it was determined that *H. peltigera* individuals had six larval stages in both safflower varieties as in *H. armigera* (Borah & Dutta, 2002). According to the safflower varieties, a statistical difference was determined between the development periods of the first and third stage larvae (P<0,05, Table 1). For other larvae, prepupa and pupa stages, development times were very close to each other in both varieties (P>0,05). However, the pre-adult time of BS took a mean of 32.82±0.23 days on Balcı and 33.97±0.16 days on Dinçer and the effect of safflower variety on the growth period of *H. peltigera* was found to be significant (P<0,05). In contrast, pre-adult survival rates are very close to each other in both varieties (0.56±0.04 on Balcı and 0.48±0.04) on Dinçer (P>0,05) (Table 1). The fact that the oil content of the Balcı is higher than the Dinçer (Arslan & Çulhan, 2020), have enabled to develop of the larvae better and faster. The difference can be seen in durations of the larvae at different stages of *Heliothis* species feeding on different host plants (Gomes, Santos, & Avila 2017; Rhudong et al, 2019). The durations of *H. armigera*'s first, third, fifth stage larvae and pupa stages were found different from each other according to the plant variety, it was very close in other larval periods (Dhandapani & Balasubramanian, 1980). Similarly, Naseri, Golparvar, Razmjou, & Golizadeh (2014) found that the development period of *H. armigera* fed on different soybean varieties ranged from 31.82±0.42 days to 37.58±0.90 days. Amer & El-Sayed (2014) noted that the pupal period of *H. armigera* fed on different plants at 26 °C and 14:10 photoperiod was not different from each other. The life span of *H. peltigera* adults was determined 10.58±0.31 days on Balcı and 9.98±0.28 days on Dinçer. It is seen that the life span of adults is not different from each other in both safflower varieties (P <0.05) (Table 1). There is no study on the adult life span of *H. peltigera* and effect of its host on the adult life span. In accordance with our study, several researchers (Naseri et al, 2014; Gomes et al, 2017) reported that the adult life of *H. armigera* was affected by different bean varieties.

Table 1. Means and standard errors (Ses) of larval durations, preadult times, and preadult survival rates of *Heliothis peltigera* on two safflower varieties.

Biological stages	Safflower varieties			
	n	Balcı	n	Dinçer
Eggs	131	3.19±0.07a	129	3.23±0.06a
L_1	121	3.24±0.07 b	115	4.17±0.09a
L_2	103	2.87±0.09 a	101	2.81±0.10a
L_3	102	2.60±0.07 a	101	2.32±0.06b
L_4	101	1.98±0.03 a	101	2±0.009 a
L_5	100	1.92±0.03 a	100	1.97±0.02 a
L_6	100	2.05±0.03 a	100	1.97±0.02 a
Prepupa	96	2.07±0.03a	100	2.01±0.01a
Pupa	80	12.82±0.18a	71	13.23±0.16a
Preadult time (d)	80	32.82±0.23 b	71	33.97±0.16a
Preadult survival rate (sa)	142	0.56±4.14 a	146	0.48±4.14 a

* See was estimated using the bootstrap technique with 100.000 resampling. The means followed by different letters in the same row were significantly different according to the paired bootstrap test based on the confidence interval of differences P <0.05.

Sex ratio, oviposition and fecundity

Other data obtained in this study was about the sex ratios on two different host plants of the pest. Pupa sex ratio (♀:♂) of *H. peltigera* was found as 1.17: 1 on Balcı, and 1: 1.19 on Dinçer. In adults, it was determined as 1.05: 1 on Balcı and 1: 1.21 on Dinçer. The findings showed that the share of females on Balcı and males on Dinçer variety in the population was higher. Similar studies with *H. armigera* show that the sex ratio favors males. Kaya & Kovancı (2000) noted that the sex ratio (♀:♂) of *H. armigera* pupae was 1.00: 1.07 and 1.00: 1.08 in adults.

It was determined the APOP, TPOP and oviposition period of *H. peltigera* females feeding with two safflower varieties were 3.24±0.20, 35.91±0.4 and 3.45±0.17 days on Balcı; 4.46±0.36 and 37.96±0.42, 3.36±0.15 days on Dinçer, respectively. The differences in APOP and TPOP of these adult females were found to be statistically significant, while oviposition periods were similar (P<0,05) (Table 2). Jha, Chi, & Tang (2012) reported that the APOP period of *H. armigera* was not affected by different bean varieties however the TPOP period of *H. armigera* was affected feeding with different bean varieties (Naseri et al, 2014) in our study.

The mean number of eggs of *H. peltigera* females were 606.89±48.09 on Balcı and 530.53±42.89 on Dinçer. The differences between the egg numbers of *H. peltigera* feeding in two safflower varieties were not statistically significant (P>0,05). Population doubling time (*DT*) shows that it is 5.13±0.17 days on Balcı, 5.79±0.25 days on Dinçer and the difference between them is significant (P<0.05). According to these results,

the initial population of *H. peltigera* on both varieties can double in a short period of about 5-6 days. Host plants influence the fecundity, survival and longevity of adults of *H. armigera* (Gomes et al, 2017).

Table 2. Means and standards errors (Ses) of the pre-oviposition period (APOP), total pre-oviposition period (TPOP), oviposition days (O_d), post-oviposition, total longevity, adult life span, fecundity (*F*), gross reproductive rate, and population doubling time (*DT*) of *Heliothis peltigera* on two safflower varieties.

Basic statistics	n	Balcı	n	Dinçer
Adult Pre-oviposition Period (APOP)	37	3.24±0.20 b	30	4.46±0.36 a
Total Pre-oviposition Period (TPOP)	37	35.91±0.39 b	30	37.96± 0.42 a
Oviposition days (O_d),	37	3.45±0.17 a	30	3.36±0.15 a
Fecundity (*F*)	37	606.89±48.09a	30	530.53±42.89 a
Adult life span	80	10.58±0.31a	71	9.98±0.28a
Total longevity	142	30.83±1.38a	146	29.87±1.39 a
Survival rate	142	0.97±3.60 a	146	0.97±3.78 a
GRR	142	293.83±41.13a	146	277.72±51.27 a
Population doubling time (*DT*) (day)	142	5.13±0.17 b	146	5.79±0.25 a

*See was estimated using the bootstrap technique with 100.000 resampling. The means followed by different letters in the same row were significantly different according to the paired bootstrap test based on the confidence interval of differences at the % 5 significal level.

Life table parameters

The population parameters R_o, *r*, *λ* and *T*, estimated using the bootstrap technique, were listed Table 3. The effect of host varieties on the net reproductive rate (R_0) of the population of *H. peltigera* was found to be insignificant (P<0,05). The highest intrinsic rate of increase (*r*) and the finite rate of increase (*λ*) of *H. peltigera* was calculated as 0.134 day^{-1} and 1.144 day^{-1} on Balcı, these values were found 0.199 day^{-1} and 1.12 day^{-1} on Dinçer respectively. It means that the females feeding on the Balcı can bring more live female offspring to the population in one day and have a more reproductive ability. According to these results, it is predicted that *H. peltigera* individuals fed on Balcı can give more female offspring to the population compared to Dinçer. Likewise, the mean generation time (T_0) of the pest fed in two safflower varieties was different from each other, it was shorter (37.5 days) on Balcı than on Dinçer (39.25 days) (Table 3). Naseri et al (2014) found the intrinsic rate of increase (*r*) of *H. armigera* was 0.115±0.009 day^{-1} - 0.142±0.001 day^{-1}; the net reproductive rate (R_0) were 177.3±6.7 - 270.1±6.7 offspring; the mean generation time (T_0) ranged from 37.03±0.05 to 44.64±0.07 days on 13 soybean varieties. Similar to the differences in the (*r*) value in our study, depending on the variety, the *r* value of *H. armigera* was 0.1135 day^{-1} for sunflower (Reddy, Rao, Rao, & Rajasekhar, 2004) and 0.1423 day^{-1} for millet (Patal & Koshyia, 1997) was determined. Basavaraj, Naik, Jagadish, & Shadakshari (2018) reported that the net reproductive rate (R_o) of *H. armigera* was 281.011 with mean length of generation (T_0) was 41.40 days on sunflowers. The

reasons for these differences result from morphological, physiological or ingredients of nutrient differences in each host species or a variety depending on the host plant variation. Additionally, the fact that the intrinsic rate of increase of *H. peltigera* is higher on Balcı with higher oil content support that this variety is more suitable in terms of reproductive ability. The life table parameters obtained indicate that the population of *H. peltigera* fed on Balcı will be higher.

Table 3. Means and standards errors (Ses) of the intrinsic rate of increase, (*r*), finite rate of increase (*λ*), net reproductive rate (R_0) and mean generation time (*To*) of *Heliothis peltigera* on two safflower varieties.

Parameters	Balcı (n:142)	Dinçer (n=146)
The intrinsic rate of increase, (*r*) (day^{-1})	0.134±4.59 a	0.119±5.123 b
The finite rate of increase (λ) (day^{-1})	1.144±0.005 a	1.126±0.005 b
The net reproductive rate (R_0) (egg/ female)	158.13±25.52a	109.01±19.71a
Mean generation time (T_0) (day)	37.49±0.34 b	39.25±0.40 a

See was estimated using the bootstrap technique with 100.000 resampling. The means followed by different letters in the same row were significantly different according to the paired bootstrap test based on the confidence interval of differences at the % 5 significal level.

When the survival rates of *H. peltigera* fed on two safflower varieties were examined, it was found that the eggs survived at the rate of 90.85% on the Balcı variety and 89.73% on the Dinçer variety (Fig. 1). The survival rates were determined on the first, second, third, fourth, fifth and sixth larval period of *H. peltigera* of fed on Balcı variety as 93.8%, 85.12%, 99.03%, 99.02%, 99.01%, and 100%, respectively. On Dinçer, the survival rates of the first, second, third, fourth and fifth larval period were recorded as 87.79%, 87.83% 100%, 100%, 99.01%, and 100%, respectively. From the third larval period to the pupal period, survival rates were high in both cultivars. Additionally, the survival rates of prepupa and pupa were 96% and 83.33%, respectively, on Balcı while prepupa was 100% and pupa was 71% on Dinçer variety. According to the age distribution, the survival rate was determined as 97.71% on Balcı and 97.45% on Dinçer (Fig. 1). Due to the variable development rate among individuals, the age-stage survival rate (sxj) showed the stage differentiation and significant stage overlaps (Fig. 1). In general, the lowest survival rate on both varieties was observed in the pupal stage. The adults emerged faster on Balcı than they did on Dinçer. According to these values, more deaths were observed in the first and second instar larvae and pupal periods compared to other biological periods. Althought the mortality rates in male and female adults are close to each other, the highest male death on Balcı variety is on the 50th day, and the highest female mortality is on the 49th day; On the Dinçer variety, the highest male death was on the 48th day and the highest female death on the 49th day (Fig. 1). It was compared with the probability of survival of *H. armigera*, as no other studies have addressed the probability of survival of *H. peltigera*. The survival probability of *H. armigera* was determined to be 32% from egg to adult on different bean varieties (Naseri et al, 2014), and 33.1% from egg to pupa on cotton (Liu, Li, Gong, & Wu, 2004). Additionally, the survival rate of *H. armigera*'s pupae was reported 85.7% on cotton (Kunjun, Yuping,

& Minghui, 1992); 92.7% on artificial diet (Amer & El-Sayed, 2014), 56% on soybean, 72% on artificial diet (Suzana, Damiani, Fortuna, & Salvadori, 2015).

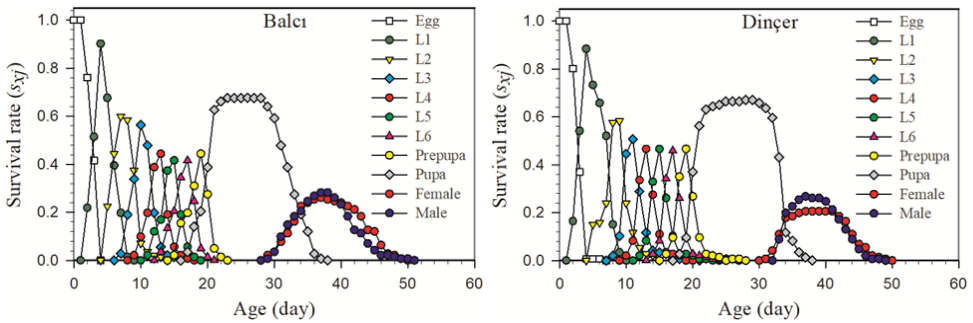

Fig. 1 Age-stage specific survival rate of *Heliothis peltigera* on two safflower varieties Sxj: the probability that a newly laid egg will survive to age x and stage j.

The life expectancy is the estimation of survival time of an individual under certain conditions. The life expectancy of a newborn individual (e_{01}) of *H. peltigera* was estimated as 31 days on on Balcı, and 30 days on Dinçer varieties. But life durations of the pest were found as almost 50 days on both host plants. When the survival rate (l_x) curve of all biological stages was examined, it was seen that the population decreased rapidly at the beginning and the survival rate decreased to 70% on the 10th day, this decline was slow until the 30th day when the adults emerged, and it decreased rapidly from the 40th to 52nd day. On the 30th day, fecundity rate (m_x) increased with the emergence of adult females and males, reaching the highest level between 36.-38 days and ended on the 44th day with the adults starting to die. It is seen that the $l_x m_x$ curve also follows a parallel course on Balcı (Fig. 3).

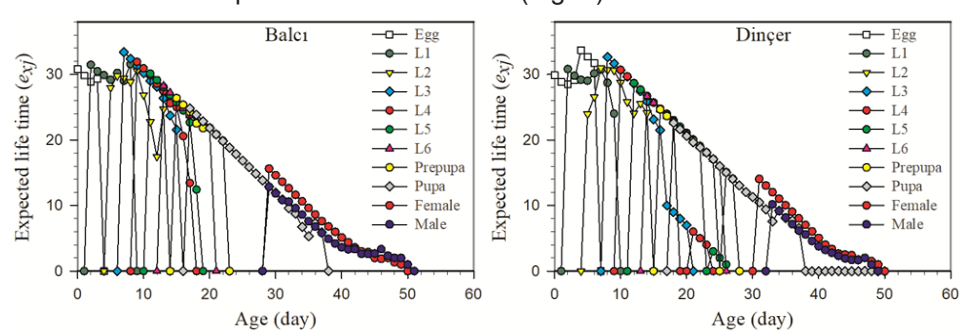

Fig. 2 Expected life time (*exj*) of *Heliothis peltigera* on two safflower varieties.

The higher reproduction values (v_{xj}) of *H. peltigera* were calculated for females on both varieties. It was estimated about 400 eggs in 33th days on Balcı, while it was found 370 eggs in 36th days on Dinçer (Fig. 4). Parallel to v_{xj}, the net reproductive rate (R_o) approaches 158.13 levels. On Dinçer, the reproductive value (v_{xj}) of the population of *H. peltigera* has increased since the 31st day and the net reproductive rate (R_o) reaches 109.01 levels. The obtained data show us that *H. peltigera* has a high reproduction capacity

as soon as it becomes an adult. The reproductive value decreases with the aging of the female, and it ends on the 45th day on the Balcı and the 47th day on the Dinçer (Fig. 5).

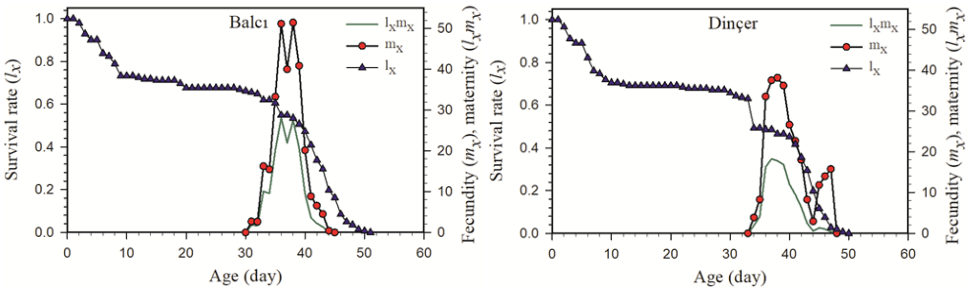

Fig 3. Population survival rate of *Heliothis peltigera* on safflower varieties Balcı and Dinçer. l x: the probability that a new egg will survive to age x. mx: the mean fecundity of individuals at age x. l x*mx: the product of l x and mx, age-stage specifc reproduction.

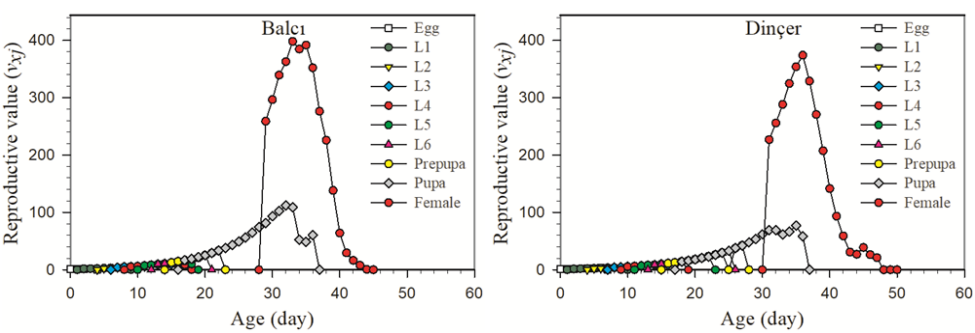

Fig.4 Reproduction value of *Heliothis peltigera* fed on two safflower varieties.

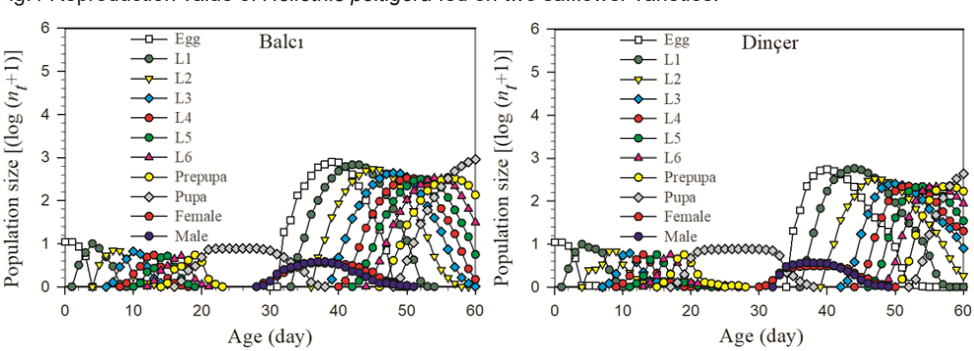

Fig.5 Population sizes of *Heliothis peltigera* on two safflower varieties.

Age-stage, two-sex life tables provide comprehensive information on the pest's population parameters. With this study, the biology and life table of *H. peltigera* in Turkey and the world was revealed for the first time and basic data were obtained about this pest. As a result, the age-stage, two-sex life table we made showed that Balcı variety was more advantageous than Dinçer for *H. peltigera*. However, to better

understand the insect-plant interaction, it is necessary to study the demographic parameters of this pest on safflower cultivars under field conditions. Additionally, the identification and extraction of secondary biochemical of safflower cultivars can significantly assist in planning more practical strategies for the management of *H. peltigera.* The basic information obtained in this study about *H. peltigera* will be useful for pest control and prefer to produce Dinçer variety that could be reducing of *H. peltigera*. With these data, the population of the pest can be estimated under field conditions and can help keep it below the economic damage threshold.

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