ONLINE ISSN 2651-3579

# Journal of the Entomological Research Society

Volume: 24 Part: 3 2022

**JOURNAL OF THE ENTOMOLOGICAL RESEARCH SOCIETY**

Published by the Gazi Entomological Research Society



**Subscription information**

Published by GERS in single volumes three times (March, July, November) per year. The Journal is distributed to members only. Non-members are able to obtain the journal upon giving a donation to GERS.

Papers in *J. Entomol. Res. Soc.* are indexed and abstracted in Science Citation Index Expanded (SCIE), Zoological Record, Biological Abstracts, BIOSIS Previews, Scopus, Essential Science Indicators, ProQuest, Scimago Journal & Country Rank (SJR), IPIndexing Portal, Resurchify, ORES, Academic Accelerator, CiteFactor, scinapse, Acarindex, Publons, TR Dizin.

Publication date: December 01, 2022

*J. Entomol. Res. Soc., 24(3): 245-255, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2134* *Online SN:2651-3579*

# Validation of IPM Modules for the Management of Whitefly, *Bemisia tabaci* and *Mungbean Yellow Mosaic Virus* Disease in Greengram

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## ABSTRACT

Greengram is known to be infected by several viral diseases. Among them, *Mungbean Yellow Mosaic Virus* (MYMV) is the most destructive in India, and it is popularly known as the yellow plague. MYMV transmitted exclusively through plant-to-plant by whitefly, *Bemisia tabaci* (Gennadius). For the management of MYMV, vector control is the only possible option. Hence, the attempt was made to evaluate IPM modules against whiteflies by reducing their population or disrupt MYMV transmission. The field experiment was conducted at Agricultural and Horticultural Research Station, Bhavikere, during *the* Kharif seasons of 2018 and 2019. The results of two seasons indicated that, the lowest whitefly population (1.70 per trifoliate leaf) and significantly lowest MYMV incidence was recorded in module-3 (Maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one spray of fish oil rosin soap @ 5ml/ liter at 40 DAS). The highest grain yield (9.64 q/ha) and cost: benefit ratio (1: 3.23) was recorded in this module, followed by module-1 (Maize as a border crop + Seed treatment with imidacloprid 600 FS @ 5 ml per kg of seeds). For the management of MYMV and its vector whitefly, *B. tabaci* module-3 was effective compared to the standard check (Dimethoate 30 EC at the rate of 1.75 ml per liter). The importance of IPM in whitefly management to reduce virus incidence is discussed.

*Keywords:* Asia-I cryptic species, Border crop, C: B ratio, maize, seed treatment, vector, virus incidence, grain yield.

Ambarish, S., Kalleshwaraswamy, C.M., Venkataravanappa, V., & Sunil, C. (2022). Validation of IPM modules for the management of whitefly, *Bemisia tabaci* and *Mungbean Yellow Mosaic Virus* disease in greengram. *Journal of the Entomological Research Society*, 24(3), 245-255.

*Received: September 11, 2021* *Accepted: October 17, 2022*

## INTRODUCTION

Greengram, [*Vigna radiata* L.] is a minor pulse crop grown worldwide, but in India, it is one of the major crop (Chatterjee & Randhawa, 1952). India is the largest producer of greengram and accounts for 54 per cent of world production, and covers 65 per cent of the world acreage (https://www.indiastat.com). Short duration and nitrogen fixation make the crop ideal for catch cropping, intercropping and relay cropping system (Lindeman & Glover, 2003). The worldwide average yield of greengram is low (389 kg/ha), and its production has not increased over the years. The main reason for the low yield is the susceptibility of the crop to biotic and abiotic stresses. Among the biotic factors, viruses are the most important group of plant pathogens affecting crop production (Sharma, Yadav, Thareja, Kaushik, & Sharma, 1993). Among the viral diseases, *Mungbean Yellow Mosaic Virus* (MYMV) causes economic losses in greengram and reducing seed yield and quality (Kang, Yeam, & Jahn, 2005). Whitefly is an important sap sucking pest that causes heavy damage to the crop through direct loss of plant vitality by feeding cells sap and by transmitting the MYMV (Banks et al, 2001). The economic losses due to this virus account for up to 85 per cent in greengram, which is spreading faster towards newer areas (Karthikeyan et al*,* 2014).

For the management of MYMV, farmers are mostly resorting to synthetic insecticides because of their quick knock-down effect with or without knowing the adverse effects. To minimize the use of hazardous chemicals, IPM strategies need to be evolved to avoid toxicity to human beings, the environment and non-target organisms. Keeping the above points in view, the present investigation was undertaken to manage whitefly vector and MYMV disease by identifying an effective IPM module.

## MATERIALS AND METHODS

### Experimental site and raising of a crop

Experiments were conducted at Agricultural and Horticultural Research Station (AHRS) Bhavikere (13°14′679″N 75°43′525″E, 567m) Tarikere, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India. The widely grown greengram variety in Karnataka, KKM-3 (Karnataka Kattalagere Mungbean-3), was sown on *Kharif* seasons of 2018 and 2019. The crop was raised by following all standard procedures according to the package of practices of University of Agricultural and Horticultural Sciences, Shivamogga, except for the plant protection measures for sucking insect pests.

### Experimental design and treatments imposition

The experiment was laid under field conditions in a Randomized Complete Block Design (RCBD) with five modules and replicated four times. The sprays of Neem Seed Kernel Extract (NSKE 5%), Fish Oil Rosin Soap [(FORS), Meenark L®, West Coast Herbochem Limited)] and Dimethoate (Tafgor® 30 EC, Rallis India) were given according to the treatment wise when the crop was 20 and 40 days old. The spraying was done manually using a hand-operated knapsack sprayer with a standard volume

of water (500 litres/ha). The treatments were implemented, as mentioned in Table 1. Greengram seeds were treated with imidacloprid (Gaucho® 600 FS, Bayers Crop Science Limited) at 5 ml per kg. The seeds were spread on the polythene sheet, and the chemical was sprinkled uniformly on seeds, mixed thoroughly by hands and shade dried for 30 minutes. Border crop maize (Hybrid: Pioneer-P3551) was selected, which was sown fifteen days before sowing of greengram.

Table 1. Details of the IPM modules evaluated against *Bemisia tabaci* and MYMV disease incidence in greengram.

| Modules | Details |
|---|---|
| Module-1 | Maize as a border crop + Seed treatment with imidacloprid 600 FS at the rate of 5 ml per kg of seeds |
| Module-2 | Maize as a border crop + Seed treatment with imidacloprid 600 FS at the rate of 5 ml per kg of seeds + Reflective ribbons |
| Module-3 | Maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one spray of Fish Oil Rosin Soap (FORS) at the rate of 5ml per liter at 40 days after sowing |
| Module-4 | Two sprays of Dimethoate 30 EC at the rate of 1.7 ml per liter at 20 and 40 days after sowing (Standard Check) |
| Module-5 | Untreated Control |

## NSKE preparation (5%)

Neem fruits were collected at UAHS, Shivamogga campus during the bearing season, and the fruits were shade dried under laboratory conditions. Five kg of neem seed kernels were grounded and powdered. The powder was soaked in 10 litres of water overnight, and then the solution was filtered through muslin cloth, and the volume was adjusted to 100 litres by adding water. Freshly prepared kernel extract was used for spraying by adding 1 per cent detergent.

## Observations on whitefly population and MYMV incidence

Whiteflies were counted in the early morning on ten randomly selected tagged plants in each treatment. The number of adult whiteflies per trifoliate leaf at 15, 30, 45 and 60 days after sowing was recorded (Ambarish, Kalleshwaraswamy, & Venkataravanappa, 2020). For effective interpretation of data, the average was calculated from all the observations of whiteflies. Similarly, MYMV disease incidence was recorded at 15 days interval by counting the number of plants showing distinct MYMV symptoms and the total number of plants in an experimental plot (for each treatment), and Per cent Disease Incidence (PDI) was worked out. To know the impact of IPM modules on MYMV per cent disease incidence, 60 days after sowing data was used for the explanation of results. Further, the data of both seasons were pooled and analyzed to bring out valuable conclusions.

## Identification of *B. tabaci* cryptic species

For identification of *B. tabaci* cryptic species, total nucleic acid from the adult *B. tabaci* collected from the experimental plot was isolated by following the method described by De Barro, & Driver, 1997. Fifty nano grams of total nucleic acid of *B. tabaci* was subjected to PCR amplification using mtCOI gene specific forward

primer (C1-J-2195-TTGATTTTTTGGTCATCCAGAAGT) and reverse primer (L2-N-3014-TCCAATGCACTAATCTGCCATATTA) (Simon, Frati, Beckembach, Crespi, Liu, & Flook 1994). Negative control was used without DNA condition in each amplification. The amplified products were cloned into the pTZ57R/T vector (Fermentas, Germany) and sequenced. The nucleotide sequence identity of mtCOI gene similarity was checked by using BLAST program available at the NCBI (National Center for Biotechnology Information, San Diego, CA, USA). Further, the mtCOI gene sequences of *B. tabaci* cryptic species were compared with sequences generated by Kankala, & Ghanim, (2019) to determine whether or not they diverged by more than 3.5%, the value these authors have reported to identify putative species genetic boundaries. The mtCOI gene sequences analysis indicated of five *B. tabaci* samples showed 98% identity among them. Hence, only one accession number (ON385112) submitted was included in phylogenetic analysis (Fig. 1). The phylogenetic analysis of mtCOI gene from the *B. tabaci* was closely clustered with *B. tabaci* cryptic species belongs to the Asia-I group.

### Confirmation of MYMV through PCR

*Mungbean yellow mosaic virus* was confirmed by visual viral symptoms and PCR using a degenerative primer to specific to begomovirus. The DNA isolated from greengram samples was amplified through PCR using primers pairs AV494/AC1048, (MYMVF-ATGGGKTCCGTTGTATGCTTG and MYMVR-GGCGTCATTAGCATAG GCAAT) as described by (Naimuddin, Akram, & Aditya, 2011). A band of approximately 1.4 kb was amplified from DNA extracted from virus infected greengram plants.

### Grain yield and cost economics

Grain yield of both the years of 2018 and 2019 were recorded on each treatment and computed to a hectare basis. The Cost: Benefit ratio was calculated based on the current price available in the market based on inputs and outputs.

### Statistical analysis

All the experimental data were transformed with the square root of $\sqrt{x+0.5}$ before analysis. Data were then subjected to analysis of variance (ANOVA), and treatments were compared by Tukey's test ($p=0.05$). The statistical analysis was performed in SPSS software (Version 16.0).

## RESULTS

### Whitefly population as influenced by different IPM modules

During *Kharif* 2018, the mean number of whitefly population varied significantly among the different IPM modules evaluated ($p<0.05$). The lowest number of whiteflies (1.80) per trifoliate leaf was recorded in module-3, which was found to be on par with module-2 (2.21) and module- 4 (2.30). The maximum number of whiteflies (3.89) per trifoliate leaf was noticed in the untreated control, and it is found to be on par with module-1 (2.68) (Table 2).

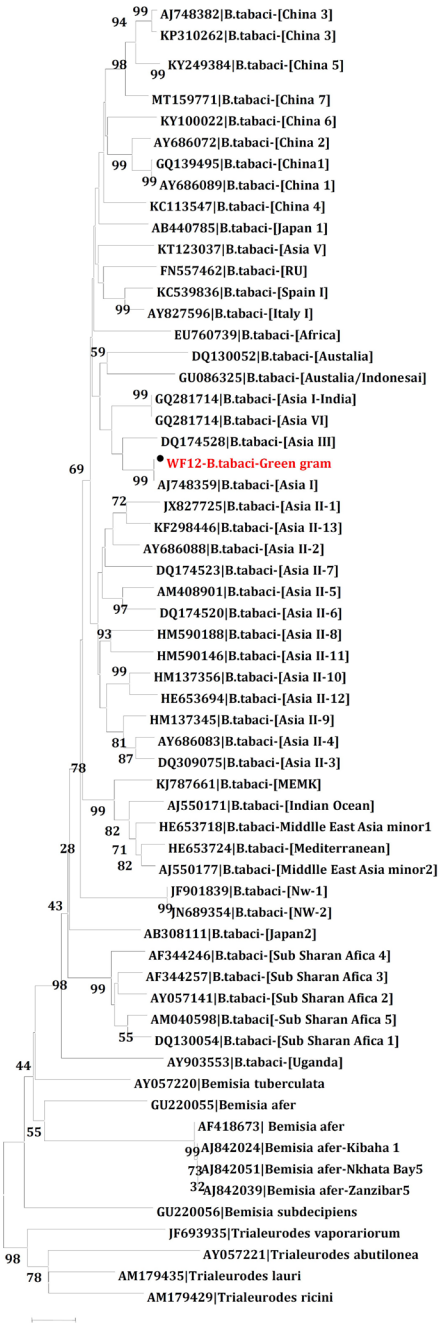


Fig. 1. Phylogenetic analysis of *B. tabaci* for confirmation of cryptic species.

Table 2. Evaluation of IPM modules against *Bemisia tabaci* on greengram during Kharif 2018.

| Modules | Mean number of whiteflies per trifoliate leaf | | | | |
|---|---|---|---|---|---|
| | 15 DAS | 30 DAS | 45 DAS | 60 DAS | Total mean |
| Module-1 | 1.80 (1.52)$^{a}$ | 2.25 (1.66)$^{b}$ | 3.10 (1.90)$^{b}$ | 3.55 (2.01)$^{c}$ | 2.68 (1.78)$^{ab}$ |
| Module-2 | 1.60 (1.45)$^{a}$ | 1.85 (1.53)$^{ab}$ | 2.50 (1.73)$^{b}$ | 2.90 (1.84)$^{bc}$ | 2.21 (1.65)$^{a}$ |
| Module-3 | 2.13 (1.61)$^{a}$ | 1.74 (1.50)$^{a}$ | 1.45 (1.40)$^{a}$ | 1.90 (1.55)$^{a}$ | 1.80 (1.52)$^{a}$ |
| Module-4 | 2.90 (1.84)$^{b}$ | 2.20 (1.64)$^{b}$ | 1.75 (1.50)$^{a}$ | 2.35 (1.69)$^{ab}$ | 2.30 (1.67)$^{a}$ |
| Module-5 | 2.85 (1.83)$^{b}$ | 3.55 (2.01)$^{c}$ | 4.25 (2.18)$^{c}$ | 4.90 (2.32)$^{d}$ | 3.89 (2.09)$^{b}$ |
| F | 16.74 | 37.57 | 64.02 | 54.40 | 6.03 |
| P | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

Numbers in the parenthesis are √(x+0.5) transformed values

DAS- Days after sowing

Mean values followed by the different letters are significantly different by Tukey's test (p≤0.05)

During 2019, a similar trend was noticed in different modules evaluated. Significantly, a lower mean number of whiteflies per trifoliate leaf (1.60) was recorded in module-3, which was found to be on par with module-4 (2.21) and module-2 (2.29). The control plot recorded the 3.63 whiteflies per trifoliate leaf, and it was found to be on par with module-1 (2.65) (Table 3).

Table 3. Evaluation of IPM modules against *Bemisia tabaci* on greengram during Kharif 2019.

| Modules | Mean number of whiteflies per trifoliate leaf | | | | |
|---|---|---|---|---|---|
| | 15 DAS | 30 DAS | 45 DAS | 60 DAS | Total mean |
| Module-1 | 1.75 (1.50)$^{ab}$ | 2.2 (1.64)$^{a}$ | 3.08 (1.89)$^{c}$ | 3.58 (2.02$^{bc}$d | 2.65 (1.77)$^{ab}$ |
| Module-2 | 1.65 (1.47)$^{a}$ | 2.0 (1.58)$^{a}$ | 2.70 (1.79)$^{bc}$ | 2.83 (1.82)$^{bc}$ | 2.29 (1.67)$^{a}$ |
| Module-3 | 1.95 (1.57)$^{abc}$ | 1.45 (1.40)$^{a}$ | 1.35 (1.36)$^{a}$ | 1.65 (1.47)$^{a}$ | 1.60 (1.45)$^{a}$ |
| Module-4 | 2.7 (1.79)$^{b}$ | 2.05 (1.60)$^{a}$ | 1.75 (1.50)$^{ab}$ | 2.35 (1.69)$^{ab}$ | 2.21 (1.65)$^{a}$ |
| Module-5 | 2.5 (1.73)$^{ab}$ | 3.4 (1.97)$^{b}$ | 4.08 (2.14)$^{c}$ | 4.55 (2.25)$^{d}$ | 3.63 (2.03)$^{b}$ |
| F | 6.47 | 9.267 | 12.86 | 30.97 | 7.11 |
| P | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

Numbers in the parenthesis are √(x+0.5) transformed values

DAS- Days after sowing

Mean values followed by the different letters are significantly different by Tukey's test (p≤0.05)

Pooled data of two seasons indicated that the lowest number of whiteflies (1.70) per trifoliate leaf was recorded in module-3, which was found to be on par with module-2 (2.25) and module-4 (2.26). In module-1 recorded 2.66 whiteflies per trifoliate leaf. The untreated control plot recorded the 3.76 whiteflies per trifoliate leaf, and it was found to be a statistically significant difference over the other modules evaluated (p<0.05) (Table 4).

Table 4. Evaluation of IPM modules against *Bemisia tabaci* on greengram (pooled data of 2018 and 2019).

| Modules | Mean number of whiteflies per trifoliate leaf | | | | |
|---|---|---|---|---|---|
| | 15 DAS | 30 DAS | 45 DAS | 60 DAS | Total mean |
| Module-1 | 1.78 (1.51)$^{a}$ | 2.23 (1.65)$^{b}$ | 3.09 (1.89)$^{b}$ | 3.56 (2.02)$^{c}$ | 2.66 (1.78)$^{ab}$ |
| Module-2 | 1.63 (1.46)$^{a}$ | 1.93 (1.56)$^{ab}$ | 2.60 (1.76)$^{b}$ | 2.86 (1.83)$^{b}$ | 2.25 (1.66)$^{a}$ |
| Module-3 | 2.04 (1.59)$^{a}$ | 1.59 (1.45)$^{a}$ | 1.40 (1.38)$^{a}$ | 1.78 (1.51)$^{a}$ | 1.70 (1.48)$^{a}$ |
| Module-4 | 2.80 (1.82)$^{b}$ | 2.13 (1.62)$^{b}$ | 1.75 (1.50)$^{a}$ | 2.35 (1.69)$^{b}$ | 2.26 (1.66)$^{a}$ |
| Module-5 | 2.68 (1.78)$^{b}$ | 3.48 (1.99)$^{c}$ | 4.16 (2.16)$^{c}$ | 4.73 (2.29)$^{d}$ | 3.76 (2.06)$^{b}$ |
| F | 17.03 | 30.43 | 42.13 | 64.79 | 5.70 |
| P | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

Numbers in the parenthesis are √(x+0.5) transformed values

DAS- Days after sowing

Mean values followed by the different letters are significantly different by Tukey's test (p≤0.05)

## MYMV disease incidence as influenced by different IPM modules

At 60 days after sowing (pooled data of two years), there was a significant difference was observed among the different modules tested (p<0.05). The lowest per cent disease incidence was documented in module-3 (7.75 %), followed by 10.65 per cent in module-2, which was found to be on par with module-1 (11.26 %). However, in standard check (module-4) recorded 14.26 per cent disease incidence. The maximum MYMV disease incidence was noticed in the untreated control plot (26.80 %) (Fig. 2). Up to 15 days of sowing, there was no disease incidence observed in module-1 and module-2 because of the effect of seed treatment (imidacloprid) and border crop (maize) effect. The overall results indicated that IPM modules had reduced the MYMV incidence in greengram.

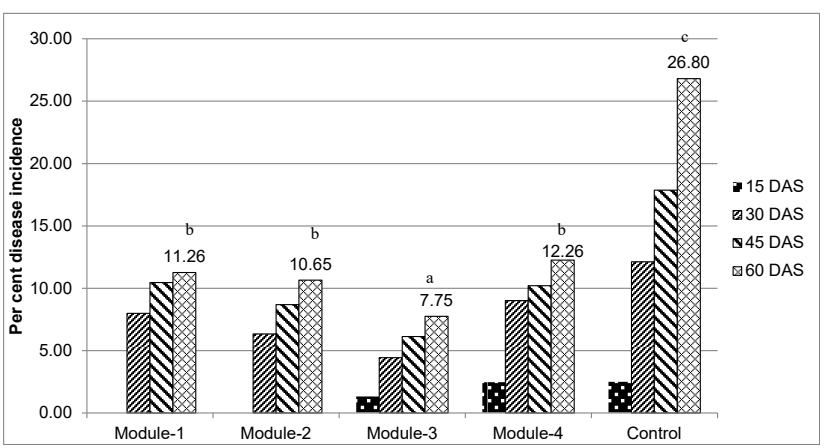


Fig. 2. MYMV disease incidence as influenced by different IPM modules in greengram (pooled data of 2018 and 2019).

**Yield and economics of greengram as influenced by different IPM modules**

Pooled data of two years (2018 and 2019) revealed that module-3 was superior among all the other treatments by documenting the highest grain yield of 9.64 q/ha. This was found to be on par with module-4 (8.93 q/ha) and module-2 (8.81 q/ha). The next best treatment with respect to yield was Module-1 (8.12 q/ha), the lowest grain yield was documented in the untreated control (6.08 q/ha) (Table 5).

Similarly, Module-3 documented the highest C: B ratio (1:3.23) and was superior when compared to other treatments, followed by module-1 (1:3.12), module-4 (1:2.94), module-2 (1: 2.89). Untreated control recorded the lowest C: B ratio (1:2.14 (Table 5).

Table 5. Impact of IPM modules on grain yield and cost economics of greengram (pooled data).

| Modules | Grain yield (q/ha) | Yield of border crop (Maize) (q/ha) | Cost of treatments for plant protection (Rs./ha) | Total Cost of cultivation (Rs./ha) | Gross income (Rs./ha) | Net income (Rs./ha) | C:B |
|---|---|---|---|---|---|---|---|
| Module-1 | 8.12$^{b}$ | 3.50 | 664 | 21 153.32 | 65 892.68 | 44 739.36 | 1: 3.12 |
| Module-2 | 8.81$^{ab}$ | 3.50 | 4 064 | 24 553.32 | 70 922.09 | 46 368.77 | 1: 2.89 |
| Module-3 | 9.64$^{a}$ | 3.50 | 3 355 | 23 844.32 | 76 971.96 | 53 127.64 | 1: 3.23 |
| Module-4 | 8.93$^{ab}$ | -- | 1 642.6 | 22 131.92 | 65 090.77 | 42 958.85 | 1: 2.94 |
| Module-5 | 6.08$^{c}$ | -- | -- | 20 489.32 | 43 879.78 | 23 390.46 | 1: 2.14 |
| F | 19.65 | -- | -- | -- | -- | -- | -- |
| P | <0.05 | -- | -- | -- | -- | -- | -- |

(Market price of greengram= Rs. 7313/quintal, Variety-KKM-3): (Market price of Maize= Rs. 1916/quintal)
Mean values followed by the different letters are significantly different by Tukey's test (P≤0.05)
Cost of production for all the modules: 20489.32 Rs./ha.

From the above results, it indicated that the module-3 (maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one spray of FORS @ 5ml/liter at 40 days after sowing) was found to be superior in reducing the whitefly population and MYMV incidence. The grain yield (9.64 q/ha) and cost economics (1: 3.23) also superior when compared to the standard check and other modules evaluated.

The next best module with respect to cost economics was module-1 (Maize as a border crop + seed treatment with imidacloprid) (1:3.12). In this module, the grain yield was low when compared to the other modules. The C: B ratio was high because the cost incurred in this module was low, and also due to the addition of border crop yield (maize yield 3.50 q/ha). Hence, module-3 and module-1 were cost-effective in managing whitefly vector and MYMV disease incidence in greengram.

## DISCUSSION

MYMV is one of the major bottle neck to greengram cultivation and production in Asia, including India. The management of this disease remains a major challenge. Currently, the outbreak of whitefly and the resistance development to most of

insecticides (Ahmad, Arif, & Naveed, 2010), and incidence of MYMV was increased in many leguminous crops in Indian conditions (Karthikeyan, Shobhana, Sudha, Raveendran, Senthil, Pandiyan, & Nagarajan, 2014). Farmers spraying three rounds of insecticides for management of MYMV hence, the IPM modules play an important role in the ecofriendly management. In the present study, different combination of components was tested against whitefly and MYMV in greengram. Among the different combinations, maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one spray of FORS @ 5ml/liter at 40 days after sowing was found to be effective. Because maize is a tall-growing plant and acts as a barrier crop that helps prevent the movement of small insect pests from one field to another, which are generally carried by the wind. Earlier reports also indicated maize as a border crop is known to reduce the whitefly and virus incidence in soybean (Raghupathi & Sabitha, 1994). Similarly, the treatment with border crop of African tall maize + seed treatment with imidacloprid 70 WS + reflective mulch + spraying of triazophos 40 EC at 0.175 % at 30 days after sowing + spraying with thiamethoxam 25 WDG at 0.05 % at 45 DAS recorded the lowest whitefly population, yellow mosaic virus incidence and highest yield in pole bean (Jyothi, Nagaraju, Padmaja, & Rangaswamy, 2013). The effectiveness of border crop and seed treatment for the management of insect pests in different pulse crops has been reported (Kumar et al*,* 2014; Anusha, Balikai, & Patil, 2016; Swathi & Gaur, 2017; Radhika, Reddy, Anitha, Vidhyasagar, & Ramesh, 2018).

Fish oil kills insects on contact by disrupting gas exchange (respiration), cell membrane function or structure. They also kill them by disrupting their feeding on oil-covered surfaces. The toxic action is more physical than chemical (Bogran, Ludwig, & Metz, 2011). The FORS was effective in many insect pests, as reported by Rahman & Batra (1945), who proved effective in controlling onion thrips. Natrajan, Sundramurthy, & Basu (1991) found that FORS was superior in controlling *Thrips tabaci* in cotton. Jha & Manoj Kumar (2018) reported that NSKE 5 % recorded the 76.02 per cent reduction in whiteflies. Similarly, Jayraj et al (1986) recorded 93.7 per cent mortality of whiteflies in NSKE treatment. Natarajan et al (1986) reported that neem oil was effective in suppressing the whitefly population. Hence, the combination of border crop and one spray of NSKE 5% and FORS has effectively reduced the deadly MYMV and its vector whitefly population in greengram. The earlier researchers also proved that IPM modules were effective in the reduction of the pest population, good grain yield and higher benefits in various pulse crops such as urdbean (Singh, Tomar, & Rikhari, 2018), french bean (Sharmah & Rahman, 2017) and pigeonpea (Gajendran, Chandrasekaran, & Jebaraj, 2006; Srinivasan & Sridhar, 2008). Various explanations have been given for the dynamics of insect pest populations under various cropping systems. Insect population reductions have been generally attributed to disruption of the insects visual and olfactory responses (Giga & Munetsi, 1989; Rizk, 2000). The companion and neighbouring plants can reduce pest pressure by providing habitat for the natural enemies, preventing entry into the field, dispersal and confuse pests (Wallace, 2013).

From the present investigation, it was revealed that IPM module consisting of maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one

spray of FORS @ 5ml/liter at 40 days after sowing was found to be effective in the management of whitefly vector and MYMV disease incidence in greengram. This module recorded the highest grain yield and C: B ratio when compared to the standard check and other modules.

## ACKNOWLEDGEMENTS

This work was supported by Director of Research, University of Agricultural and Horticultural Sciences, Shivamogga-577204, Karnataka, India through Staff Research Project.

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*J. Entomol. Res. Soc., 24(3): 257-268, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2160* *Online ISSN:2651-3579*

# Evolution of the Larvicidal Effects of Secondary Metabolites Produced by *Bacillus subtilis* (Ehrenberg, 1835) Cohn, 1872 Wild Type UTB1 and Mutant M419 against *Plutella xylostella* (L., 1758) (Lep.: Plutellidae)

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## ABSTRACT

The effects of *Bacillus subtilis*, wild type bacterium UTB1 and mutant M419, on two larval instar groups of the diamondback moth, *Plutella xylostella* L. (Lep.: Plutellidae) until three days after treatment have been investigated. Larval mortality rate increased with increasing the concentration and over the days after treatment. Increasing the larval instar resulted in elevating the $LC_{50}$ value for both types of biosurfactants. In terms of lethality, there was no significant difference between the two bacteria in the final mortality rate after three days.

*Keywords: Plutella xylostella*, biosurfactant, *Bacillus subtilis*, larvicidal effect, biology, *Brassicaceae.*

Tajdini, M., Osouli, S., & Afsharmanesh, H. (2022). Evolution of the larvicidal effects of secondary metabolites produced by *Bacillus subtilis* (Ehrenberg, 1835) Cohn, 1872 wild type UTB1 and mutant M419 against *Plutella xylostella* (L., 1758) (Lep.: Plutellidae). *Journal of the Entomological Research Society*, 24(3), 257-268.

*Received: October 19, 2021* *Accepted: October 17, 2022*

## INTRODUCTION

At present, food security and the sustainable development of agriculture are among the most important national priorities in Iran. However, the agricultural sector faces significant problems and challenges to achieve these objectives. The most significant challenges in this area include living and non-living environmental stresses such as pests and diseases, drought, salinity, high production costs, low quality of crops, environmental pollution caused by the usage of pesticides and chemical fertilizers and the high amount of agricultural wastes (Nematollahi & Bagheri, 2006).

The Brassicaceae family includes 10 tribes, 350 genera, and about 400 species. The most important member of this family is *Brassica oleracea* (cabbage), which has about 20 different varieties (Nematollahi & Bagheri, 2006). Also, there is a wide range of ornamental plants in this family which have many applications in food, industrial and medicinal areas (Avato & Argentieri, 2015).

One of key pests of the *Brassicaceae* family in Iran is the diamondback moth with the scientific name of *Plutella xylostella* L. This pest which belongs to the Lepidoptera order and the Plutellidae family causes most of the damage to *Brassicaceae* crops (Nematollahi & Bagheri, 2006). Feeding these species' larvae causes cabbage crop damage. Although the pest's larvae are very small in size, they can completely remove the leaf tissue except for veins. This is especially destructive to seedlings which may cause inflorescences cutting-off on cabbage, cauliflower, and broccoli (Knodel & Gaehiarachchi, 2008).

In many countries, the diamondback moth has become resistant to many artificial insecticides. Also, this moth has shown resistance to DDT and continued success of the wide utilization of *Bacillus thuringiensis* (Bt) is threatened by the development of resistance in this pest (Tabashnik, 1994; Sarfaraz & Keddie, 2005). Given that in the past decade chemical insecticides and chemical fertilizers were removed from the market in modern agriculture due to their hazardous effects on the natural environment, the biological control agents including effective microorganisms and bacterial products have recently attracted attention, instead of chemicals (Ghribi et al, 2012a).

Among the beneficial bacteria, the species of the genus *Bacillus* are known as biological pesticides to protect plants. *Bacillus* is a large and diverse genus of about 50 species from a family called Bacillaceae. The bacterium *Bacillus subtilis* was named in 1972 by Ferdinal Cohn. These bacteria are rod-shaped, gram-positive, obligate aerobic or facultative anaerobic and catalase-positive. Also, they produce endospores that can tolerate adverse environmental conditions. *Bacillus subtilis* bacteria are considered saprophytes in soil and vegetables (Alcazar-Fouli, Mellado, Alastruey-Izquierdo, Cuenca-Estrella, & Rodriguez-Tudela, 2008).

The advantage of *Bacillus* species is the production of a wide range of secondary metabolites with antimicrobial and pesticide activity including biosurfactant compounds (Ghribi et al, 2012; Kim, Ryu, Kim, & Chi, 2010). Biosurfactants have no toxic or allergic effects on humans or animals and can replace chemical pesticides (Quentin, Besson, Peypoux, & Michel, 1982).

Among these compounds, amphiphilic cyclic lipopeptides are of particular importance which are divided into three families, depending on their amino acid structure: iturins, fengycins, and surfactins (Ongena & Jacques, 2008).

Biosurfactant lipopeptides produced by *B. subtilis* are known as biocontrol compounds for disease reduction and plant pest control. *B. subtilis* UTB1, a biocontrol bacterium isolated from Iranian pistachio nuts, had shown antagonistic activity against aflatoxin-producing *Aspergillus flavus* R5 (Farzaneh & Nikkhah, 2012). This strain produces lipopeptide compounds iturins, fengycins and surfactins but the amount of these lipopeptides is not considerable (Afsharmanesh, Ahmadzadeh, Javan-Nikkhah, & Behboudi, 2014). Mutant *B. subtilis* M419 was created by random mutagenesis using gamma irradiation on strain UTB1. Production of lipopeptides surfactin, fengycin and iturin families increased in M419 (Afsharmanesh et al, 2014). M419 has shown inhibitory and mortality effects against both *Aspergillus flavus* as a fungal species and *Papillio demoleus* L. as an insect species (Afsharmanesh et al, 2014; Osouli & Afsharmanesh, 2016) which can make it an appropriate candidate for environmentally friendly bio pesticide and commercial product.

Considering the growing population of the diamondback moth and the ineffectiveness of numerous insecticides available, the present study attempts to provide a solution as one of the safe biological control methods. In this study, we assessed larvicidal effect of lipopeptides of strain *B. subtilis* UTB1 and *B. subtilis* mutant M419 on mortality of *P. xylostella* and compared them together. Mutant M419 bacterium produces more biosurfactant lipopeptides than its wild type UTB1; so, if they have at least the same effect in terms of larval mortality, using mutant M419 is recommended to produce high volumes of biosurfactants.

## MATERIALS AND METHODS

### Collecting the diamondback moths

After analyzing *Brassicae oleracea* cabbage field of Alborz province (35.9960° N, 50.9289° E), infested cabbage with different biological stages of the diamondback moth, were collected. The collected samples were placed in plastic containers and transferred to the laboratory.

### Insect laboratory rearing

The walk-in incubator by 3×4×3 m dimensions was used to rear insects at 26±2°C under 16:8 hours photoperiod and 65±5% relative humidity. Fifty couples of males and female adults were confined in the fiber glass oviposition cages with the dimensions 50×50×50 cm. A square hole was designed inside each wall of the cages and covered with mesh cloths for ventilation. The cages contained 10% sucrose solution to feed the adult insects and red cabbage for egg-laying (because of color contrast with the white eggs). Red cabbages including eggs were collected daily from the cages and transferred to plastic containers (30×25×20 cm) with some holes (covered by mesh clothes) in their walls for ventilation. Hatched larvae were kept in these containers until

the last growth stage and fresh cabbage put inside the container as their food every other day. The last-age larvae were transferred to the petri dishes and conveyed to the oviposition cage after entering the pupal stage. Voucher specimens of *P. xylostella* from this study were deposited in the insect collection of department of entomology, Tarbiat Modares University, Tehran, Iran.

**Providing and storage of bacterial isolates**

The antagonist bacterium *B. subtilis* UTB1 which were (isolated from pistachio green skin), was received from Department of Plant Protection, University of Tehran, Tehran, Iran.

The mutant bacterium *B. subtilis* M419 obtained by gamma irradiation (2 KGy dose) from the parental bacterium *B. subtilis* UTB1, in Nuclear Agriculture Research School at Alborz Health and Agricultural Research Center. The research conducted by Afsharmanesh et al (2014) showed that the biological control of mutant M419 against *A. flavus* was significantly increased compared to wild type strain UTB1. For long-term storage of bacterial strains, a 16-hour culture medium of bacteria in the nutrient broth (NB) was mixed with 30% sterile glycerol in Eppendorf vials and, then transferred to a freezer at -70°C.

**Cultivation conditions of bacterial isolates**

At first, a colony of UTB1 and M419 bacteria were grown in 5 mL of NB medium at 30°C in a shaker incubator and then inoculated into 50 ml of medium for producing cyclic lipopeptides called MOLP (optimal medium for lipopeptides production) which were grown for three days in a shaker incubator at 30°C (Ahimou, Jacques, & Deleu, 2000). Then, the supernatants were separated by centrifugation at 10,000xg and 4°C for 15 minutes.

**Extraction of cyclic lipopeptides from bacterial isolates**

Cyclic lipopeptides were extracted from the supernatants of UTB1 and mutant M419 in three steps by adding a quarter (vol) of normal butanol solvent and vigorous shaking for 60 min on a shaker in the first step and 15 minutes in the second and third steps based on the method described by Yazgan, Ozcengiz, & Marahiel (2001). After each step, the organic phase was separated from the inorganic phase by centrifugation at 10000xg for two minutes, and butanol layers were collected in a separate container. The butanol layer was completely evaporated under laboratory fume hood in room temperature. The remaining residues were collected, and their weights calculated. Then, different concentrations of the extracted lipopeptides were prepared, and their effects on the mortality of *P. xylostella* larvae were investigated.

**Bioassay experiment**

To study the biological factors (developmental period of each immature stage, the mortality of each stage, total number of eggs laid, percentage of eggs hatching), the eggs with the age interval of 0-24 hours were taken from adult insects. For this purpose,

each pair was placed in small cylindrical containers (15×7 cm) covered with meshes and the pest-free red cabbage leaves were supplied for 24 hours for oviposition of females. The experiments were performed on different instars of the diamondback moth larvae which were kept hungry for 5 hours. To perform these experiments, due to the results of previous studies which show that larvae with instar (1 - 2) and (3 - 4) are different in terms of resistance to control methods, the experiments performed in two stages for young and more developed larvae. For this purpose, pre-tests were designed with doses of 1000, 1500, 2000, 2500 and 3000 mg/l. According to the results of initial experiments, the main experiments were designed in the dose range of 1500 to 3500 µg/ml and 3500 to 9500 with logarithmic intervals of doses for young and more developed larvae, respectively. Thus, the young larvae were placed in Petri dishes on leaves that were immersed in previously determined concentrations (2850, 2300, 1850, 1500 and 3500 µg/ml) from UTB1 and M419 biosurfactants and dried in ambient air. After 24, 48, and 72 hours, larval mortality was recorded daily in each petri dish.

In the next step, the old larvae were tested. The same experiment was performed with more developed larvae at concentration of 3500, 4500, 6000, 7500 and 9500 µg/ml) of UTB1 and M419 biosurfactants. This experiment was conducted in triplicate with 50 larvae for each replication.

### Data analysis

In this study, the mortality doses ($LC_{50}$ and $LC_{90}$) were calculated for each experiment by probit analysis using SPSS18 software. The results of the mortality rate of two different bacteria (UTB1 and M419) were measured by split plots based on the randomized complete block design with three replications. The normality and homoscedasticity assumptions were carried out using SPSS 18. The mean values were compared by Tukey's test. All the graphs were drawn using Microsoft Excel version 2019.

## RESULTS

### Larvicidal effect of *B. subtilis* UTB1 and M419 biosurfactants against two larval age groups of *P. xylostella*

The results showed that $LC_{50}$ of UTB1 and M419 bacteria for larvae instar 1-2 of the diamondback moth at the end of initial 3 days after the exposure were 2390.303 and 2607.124 µg/ml, respectively. Also, $LC_{50}$ of these bacteria was calculated on larvae instar 3-4 which was measured as 5764.964 and 6149.872 µg/ml for UTB1 and M419 bacteria at the end of third day after the treatment, respectively. The $LC_{90}$ of bacteria UTB1 and M419 were also calculated for both larval age groups. The obtained values were 4706.520 µg/ml at lower ages and 13412.563 µg/ml at higher ages for UTB1, and 5747.513 µg/ml at lower ages and 15048.042 µg/ml at higher ages for M419, respectively (Table 1).

According to these results, $LC_{50}$ and $LC_{90}$ values for both bacteria increased with increasing larval age. Also, these values significantly decreased over post-exposure days.

Table 1. $LC_{50}$ and $LC_{90}$ of *Bacillus subtilis* UTB1 and M419 biosurfactant, against two larval age groups of *Plutella xylostella*, 1, 2 and 3 days after contact.

| Lethal concentration LC (µg/ml) | Larval instar | Bacillus subtilis | Days after contact | | |
|---|---|---|---|---|---|
| | | | 1st | 2nd | 3rd |
| 50 | 1-2 | UTB1 | 4260.618 | 2627.884 | 2390.303 |
| | | M419 | 4343.449 | 2993.162 | 2607.124 |
| | 3-4 | UTB1 | 9719.804 | 7020.316 | 5764.964 |
| | | M419 | 10429.007 | 7366.478 | 6149.872 |
| 90 | 1-2 | UTB1 | 13150.643 | 5624.671 | 4706.520 |
| | | M419 | 8697.090 | 6151.343 | 5747.513 |
| | 3-4 | UTB1 | 22247.278 | 16884.868 | 13412.563 |
| | | M419 | 20775.247 | 16370.502 | 15048.042 |

## $LT_{50}$ value for different applied concentration of UTB1 and M419 biosurfactants on two larval age groups of *P. xylostella*

Table 2 shows $LT_{50}$ value for each applied concentration of UTB1 and M419 biosurfactants on the diamondback moth larvae aged 1-2$^{th}$ and 3-4$^{th}$ instars. This value for younger larvae exposed to UTB1 was measured as 45.239 and 1.201 days at 1500 and 3500 µg/ml (lowest and highest applied doses) and as 11.057 and 1.581 days for M419, respectively. Measured $LT_{50}$ in the concentrations of 3500 and 9500 µg/ml (lowest and highest doses applied) on older larvae was 12.350 and 1.096 days for UTB1 and 20.481 and 1.143 for M419. The results indicated that the required time causing a 50% mortality rate in larvae of both age groups are significantly reduced with increasing doses. The results also revealed that the larval mortality of first group, at two doses of 2850 and 3500 µg/ml for UTB1 and only at dose of 3500 µg/ml for M419 reached 50% up to 3 days after exposure and this value reach 50% at the doses of 6000, 7500 and 9500 µg/ml for UTB1 and 7500 and 9500 µg/ml for M419 on older larval group, up to 3 days after exposure. The feeding ability of larvae decreased with increasing doses and the change in the body color was also evident.

Table 2. $LT_{50}$ value for different applied concentration of UTB1 and M419 biosurfactants on two larval age groups of *Plutella xylostella.*

| Larval instar | Bacillus subtilis | $LT_{50}$ for each concentration (days) | | | | |
|---|---|---|---|---|---|---|
| 1-2 | Concentration (µg/ml) | 1500 | 1850 | 2300 | 2850 | 3500 |
| | UTB1 | 45.239 | 15.644 | 3.113 | 2.611 | 1.201 |
| | M419 | 11.057 | 7.386 | 4.234 | 4.006 | 1.581 |
| 3-4 | Concentration (µg/ml) | 3500 | 4500 | 6000 | 7500 | 9500 |
| | UTB1 | 12.350 | 5.192 | 2.847 | 2.132 | 1.096 |
| | M419 | 20.481 | 4.794 | 4.041 | 2.488 | 1.143 |

## Dose-response lines for the larval mortality of *P. xylostella*

The effect of *B. subtilis* UTB1 and M419 against the diamondback moth larvae in two different age groups, on days 1, 2, and 3 after treatment are shown in Figs. 1, 2,

3 and 4, as the response of probit dose line. The estimated dose line for each day is drawn, and the observation points are placed around this line. Based on the results, the larvicidal effect of biosurfactant of these bacteria increased by increasing the doses and during the days after treatment.

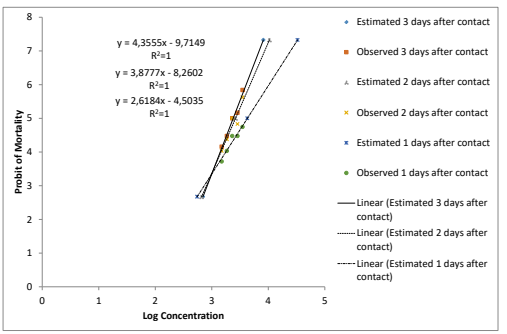


Fig. 1. Dose-response line for the younger larval mortality (1 and 2 instars) of *Plutella xylostella* after applying UTB1 biosurfactant.

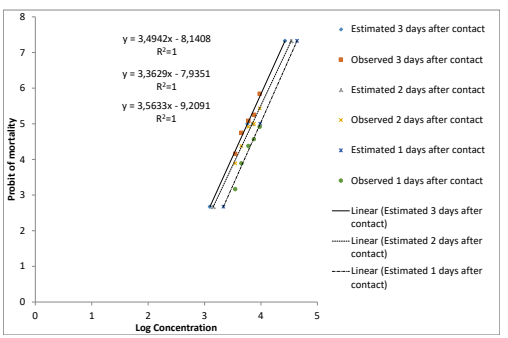


Fig. 2. Dose-response line for the older larval mortality (3 and 4 instars) of *Plutella xylostella* after applying UTB1 biosurfactant.

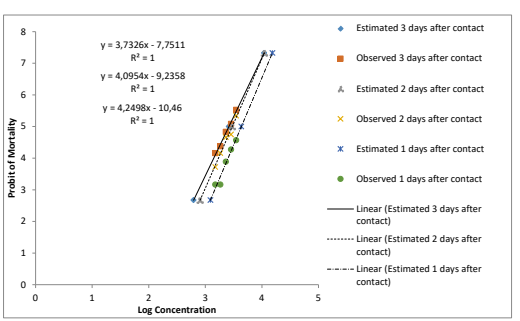


Fig. 3. Dose-response line for the younger larval mortality (1 and 2 instars) of *Plutella xylostella* after applying M419 biosurfactant.

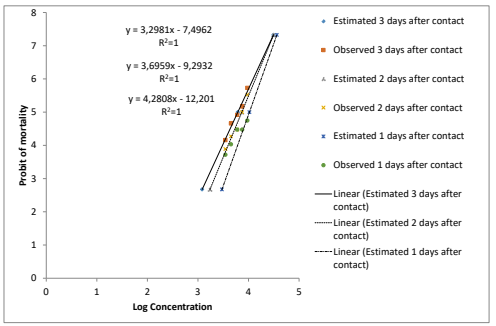


Fig. 4. Dose-response line for the older larval mortality (3 and 4 instars) of *Plutella xylostella* after applying M419 biosurfactant.

## Analyzing variance of the diamondback moth larval mortality rate after applying different doses of UTB1 and M419 bacteria

Table 3 and 4 show the results of the analysis of variance of different doses of UTB1 and M419 bacteria on the diamondback moth larvae instars 1-2 and 3-4, 3 days after exposure, respectively. Based on the results of the analysis of variance, the effects of doses of UTB1 and M419 on the mortality rate of both larval instar groups were significant, but the effects of bacterial type and the interaction between dose and bacterial type were not significantly different.

Table 3. Analyzing variance of the mortality rate of the diamondback moth larvae instar 1-2 after application of different doses of UTB1 and M419 biosurfactants.

| Source | Degree of freedom | Mean Square |
|---|---|---|
| Replication | 2 | 243.333 |
| Concentration | 4 | 3046.667** |
| Error | 8 | 64.167 |
| Bacterial type | 1 | 213.333 |
| Concentration × Bacterial type | 4 | 13.333 |
| Error | 10 | 73.333 |
| Total | 30 | |

**Significant at 1% probability level (P≤0.01, F=41.545)

Table 4. Analyzing variance of the mortality rate of the diamondback moth larvae instar 3-4 after applying different doses of UTB1 and M419 biosurfactants.

| Source | Degree of freedom | Mean Square |
|---|---|---|
| Replication | 2 | 163.333 |
| Concentration | 4 | 3158.333** |
| Error | 8 | 88.333 |
| Bacterial type | 1 | 83.333 |
| Concentration × Bacterial type | 4 | 8.333 |
| Error | 10 | 43.333 |
| Total | 30 | |

**Significant at 1% probability level (P≤0.01, F=72.885)

## DISCUSSION

*Plutella xylostella* larvae attack the leaves of cruciferous vegetables and cause severe damage. This pest has shown significant resistance to almost every synthetic insecticide applied in the field. In the present research, the effects of the biosurfactant secreted by *B. subtilis* UTB1 and M419 strains, as an ecofriendly method for crop protection against the cabbage moth were investigated in comparison with each other. UTB1 strain was isolated from Iranian pistachios and has the ability to secrete lipopeptide compounds. M419 mutant strain has the ability to produce more amounts of biosurfactants compared to UTB1 and in this regard is more suitable for commercial products.

In the present study, the effects of different doses of biosurfactant of UTB1 and M419 *B. subtilis* bacteria in the range of 1500-9500 µg/ml on the mortality rate of the diamondback moth larvae of different instars were measured to estimate the minimum suitable dose for controlling of this insect, for the first time. $LC_{50}$ and $LC_{90}$ values were measured after 1, 2 and 3 days of exposure. The results showed that the mortality rate of larvae increased by increasing doses and days after exposure of both UTB1 and M419 bacteria. The tolerance to bacterial biosurfactant doses increased by larval instars. Based on the results, there was no significant difference between UTB1 and M419 bacteria in causing final larval mortality after 3 days.

Several published studies have described mortality effects of *B. subtilis* biosurfactant on lepidopteran larvae. Osouli and Afsharmanesh (2016) measured the effect of biosurfactant of *B. subtilis* mutant M419 on the mortality rate of *Papilio demoleus* L. (Lep.: Papilionidae) larvae in the first and second instars using leaf immersion in doses of 450, 900, 1800 and 3600 mg/l in the laboratory. $LC_{50}$ and $LC_{90}$ values were measured after four days of exposure, and the results showed that the mortality rate of larvae increased by increasing doses of bacterial biosurfactants and also over days after exposure. The crude biosurfactant retained the larvicidal activity even when autoclaved at 121ºC for 15 min. M419 mutant could significantly inhibit the growth of *Aspergillus favus* in cross-culture experiments.

Ghribi et al (2012a) investigated the toxic effects of biosurfactant *B. subtilis* $SPB_1$ against *Spodoptera littoralis* (Boisduval) (Lep.: Noctuidae) neonate larvae. Lipopeptide biosurfactant displayed toxicity with an $LC_{50}$ of 251 ng/cm$^2$ with 95% confidence limits of (195-307 ng/cm$^2$). The treatment effect with different concentrations of SPB1 biosurfactants was manifested in reducing adult emergence and prolongation of the maturity period. Larval survival rate and success in entering the pupal stage increased by decreasing the concentration; meanwhile, no mortality was observed in control treatment (buffer solution).

The insecticidal activity of lipopeptid biosurfactant of *B. subtilis* SPB1against *Ectomyelois ceratoniae* (Zeller) (Lep.: Pyralidae), was investigated by Mnif, Elleuch, Chaabouni, & Ghribi (2013). The $LC_{50}$ and $LC_{90}$ values after six days of contact were measured as 152.3 and 641 µg/g, respectively. The mean lethal dose measured on four and five days after treatment was much higher than that obtained after six days of treatment, indicating the effect of time on mortality and their results represented a strong positive correlation between the applied doses and larval mortality.

There are reports on larvicidal effects of biosurfactant extracted by *B. subtilis* strains on mosquito species. The study on the effects of *B. subtilis* metabolites on mosquito larvae showed that *B. subtilis* isolated from soil specimens killed larvae of the *Aedes aegypti* (Lineaus) (Dip: Culicidae). The results indicated that the mortality rate was dose-dependent for all instars of larvae. The $LC_{50}$ and $LC_{90}$ values were determined as 1.73 and 3.71 µg/ml, respectively after 24 h of contact indicated that secondary metabolites of *B. subtilis* had high larvicidal activity against *A. aegypti* and larval survival was significantly reduced at all examined concentrations. A significant reduction in the activities of acetylcholinesterase, α-carboxylesterase, and acid phosphatases were recorded in larvae exposed to the metabolite (Revathi et al, 2013).

In the study by Das & Mukherjee (2006), larvicidal potency of cyclic lipopeptides (CLPs) secreted by two *B. subtilis* DM-03 and DM-04 strains against third instar larvae of *Culex quinquefasciatus* Say (Dip: Culicidae) were recorded. $LC_{50}$ of the crude CLPs were determined as 120.0 ± 5.0 and 300.0 ± 8.0 mg/l respectively, post 24 h of treatment.

In the present study, feeding ability of larvae decreased with increasing doses and reached zero three days after treatment and the change in the body color was evident. These effects indicated the deteriorative effect of bacterial metabolites on the midgut and digestive cells of the larvae, which caused the mature larvae to be unable to enter the pupal stage due to loss of feeding ability and they remained in the larval stage without feeding until they died.

Studies on the histopatological effects of *B. subtilis* biosurfactants on the insect midgut cells revealed the lethal changes at the cellular level. Vesicale formation in the apical of cells, lysis vacuolization of columnar cells and destruction of epithelial cell and their boundaries in the midgut larvae of *E. ceratoniae* (Mnif et al, 2013) and *S. littoralis* (Ghribi et al, 2012a) were represented. Also, according to Ghribi, Elleuch, Abdelkefi-Mesrati, Boukadi, & Ellouze-Chaabouni (2012b), the tested dose level of *B. subtilis* SPB1 caused strong histopathological disturbances in the midgut of *Ephestia kuehniella* Zeller (Lep: Pyralidae). The most frequents of which were recorded as cell vacuolization, microvilli damage and epithelium cell content passing into the midgut lumen.

Despite increasing mortality over the days after treatment in our study, the mortality rate of larvae instar 1-2, after three days of treatment with biosurfactants UTB1 and M419 in concentrations less than 2300 and 3500 µg/l, still did not reach 50%. Also, the mortality rate for larvae instar 3-4 of this insect, for two bacteria UTB1 and M419, at doses lower than 2300 and 2850 µg/l, respectively could not create 50% mortality either. In general, the results indicated that the impact rate of UTB1 bacteria was higher than M419, and at similar doses, a shorter time was required to cause 50% mortality in the larval population of both groups.

In the research by Osouli & Afsharmanesh (2016), the effect of *B. subtilis* biosurfactant M419 was calculated after 2, 3 and 4 days of the exposure to *P. demoleus* L. As the dose increased, the mortality rate increased, and $LT_{50}$ for doses of 1800 and 3600 mg/L was 2.48 and 2.841 days, respectively. Also, the mortality rate of larvae after four days at concentrations of 4500 and 900 did not reach 50%.

## CONCLUSION

The biosurfactants of UTB1 bacteria and mutant M419 could produce larvicidal effects at appropriate concentrations. Larval mortality rate increased with increasing the concentration and over the days after treatment. Increasing the larval instar resulted in elevating the $LC_{50}$ value for both types' biosurfactants, which indicated higher resistance in older larvae. In terms of lethality, there was no significant difference between the two bacteria in the final mortality rate after three days. However, the speed of biosurfactant effect of UTB1 was higher than that of M419 at similar doses, resulting in more days after treatment to cause 50% mortality in larvae of the same instar. Assuming that larvae's lethality three days after treatment is considered as an acceptable result, mutant M419 can be used on a large scale due to higher biosurfactant production. According to these results, accurate analysis of the chemical components of the metabolite secreted by the bacterium M419 compared to UTB1 and evaluating their effects on more pest species seems to be necessary.

## ACKNOWLEDGMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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*J. Entomol. Res. Soc., 24(3): 269-279, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2165* *Online ISSN:2651-3579*

# First Record of the Predatory Insects, *Androthrips ramachandrai* (Karny) (Thysanoptera: Phlaeothripidae) and *Montandoniola indica* Yamada (Hemiptera: Anthocoridae) on the Pest Thrips, *Gynaikothrips ficorum* (Marchal) (Thysanoptera: Phlaeothripidae) in Turkey

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## ABSTRACT

The Cuban laurel thrips, *Gynaikothrips ficorum* (Marchal) (Thysanoptera: Phlaeothripidae), is widely distributed to the eastern Mediterranean region of Turkey and causing damage to *Ficus microcarpa var. nitida* plants. Biological control of *G. ficorum* has a crucial role to maintain the population density of this pest insect at an acceptable level on *Ficus* trees. For this reason, to determine the potential biological control agents of this pest, survey studies have been done in Adana and Mersin provinces. The *Androthrips ramachandarai* (Karny) (Thysanoptera: Phlaeothripidae), and the *Montandoniola indica* Yamada (Hemiptera: Anthocoridae) were reported for the first time in Turkey in 2020 on *G. ficorum* that damaging young leaves of the *F. microcarpa*. Brief diagnoses of *G. ficorum* and both predatory species were provided. A total of 249 adults of *A. ramachandrai* and 461 individuals of *M. indica* were detected in the galled leaves infested with *G. ficorum*. Moreover, 1959 adults, 1335 immatures and 11851 eggs of *G. ficorum* were also recorded. There was a positive relationship between pest eggs-*A. ramachandrai* adults and pest larvae-*M. indica* adults. Ratios of prey/predatory insects indicated that the predators might have the capability in suppressing the pest thrips on *Ficus* trees.

*Key words: Ficus*, predator, relationship, thrips, Turkey.

Pehlivan, S., Atakan, E., Achirit, T.D., & Yamada, K., (2022). First record of the predatory insects, *Androthrips ramachandrai* (Karny) (Thysanoptera: Phlaeothripidae) and *Montandoniola indica* Yamada (Hemiptera: Anthocoridae) on the pest thrips, *Gynaikothrips ficorum* (Marchal) (Thysanoptera: Phlaeothripidae) in Turkey. *Journal of the Entomological Research Society,* 24(3), 269-279.

Received: October 26, 2021 Accepted: April 04, 2022

## INTRODUCTION

Following the extensive damage of palm trees (*Phoenix canariensis* hort. ex. Chabaud) by the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) to in Adana and Mersin provinces, numerous trees were stubbed, and then considerable numbers of *F. microcarpa* trees have been planted in both cities in the past decade (Atakan & Pehlivan, 2019). With the trade of the *Ficus* trees and an increase in the number of the host plants of Cuban laurel thrips, *Gynaikothrips ficorum* (Marchal) (Thysanoptera: Phlaeothripidae), which was recorded for the first time on *Ficus* sp. in Mersin Province, Turkey (Toros & Tunç, 1988), has widely distributed to the region (Atakan & Pehlivan, 2019) recently. This species is a monophagous, gall-forming thrips (Denmark, Thomas, & Funderburk, 2005) and heavily causes damage to various ornamental figs, *Ficus* spp. (Moraceae), throughout the tropical and temperate climates (Paine, 1992). This thrips reduce the photosynthetic activity of the plants by inducing galls and depreciate their ornamental value due to curled and discolored leaves (Piu et al, 1992).

Control of the pest thrips species mostly relies on using synthetic insecticides (Held & Boyd, 2008). However, applying insecticides is restricted because *Ficus* spp. has decorated both outdoor and indoor landscapes in which these are the people's living areas. Moreover, the use of broad-spectrum insecticides can develop pest resistance and affect the role of natural enemies (Arthurs, Chen, Dogramaci, Ali, & Mannion, 2011; Tavares, Torres, Silva-Torres, & Vacari, 2013). A strong association has been reported with the predators including the thrips egg predator *Androthrips ramachandrai* (Karny) (Thysanoptera: Phlaeothripidae), hemipterans such as *Montandoniola confusa* (=previously identified as moraguesi in some areas) Streito & Matocq, *Montandoniola indica* Yamada (Hemiptera: Anthocoridae). Gall formed by thrips represents a microcosm that hosting all stages of pest thrips and their natural enemies (Paine, 1992; Boyd & Held, 2006; Cambero-Campos, Valenzuela-García, Carvajal-Cazola, Rios-Velasco, & García-Martínez, 2010; Arthurs et al, 2011; Yamada, Bindu, Nasreem, & Nasser, 2011; Tavares et al, 2013).

Although many studies reported the interactions between *G. ficorum* and predatory insects, until this time there is no record from Turkey. With this study, descriptions of the predatory thrips, *A. ramachandarai* (Karny), and the hemipteran predator, *M. indica*, first recorded in Turkey were given. Additionally, the mean and total numbers of pest thrips and predatory insects and their interactions on the *Ficus* trees were summarized.

## MATERIAL AND METHODS

### Surveying area and insect sampling

Three different sampling locations were chosen for surveys of insects inhabited trees of the *Ficus microcarpa var. nitida* in outdoor landscapes of Adana and Mersin Provinces, Turkey on August 2020. I: University Campus (37°03'41.3"N, 35°21'30.7"E)

which is a natural habitat, and *Ficus* trees are grown together with citrus (*Citrus aurantium*) as ornamental; II: Adana City Center (36°59'48.8"N, 35°19'23.7"E) urban area; III: Mersin Province, Turkey (Seaside) (36°05'48.4"N, 33°01'52.7"E) in which woody and herbaceous ornamental plants are grown together. Both Adana and Mersin Provinces are located in the eastern Mediterranean region of Turkey. Mersin Province is about 80 kilometers far from Adana city, and it is a seaside province. Both cities have a typical Mediterranean climate. Ornamental arboreal plants are grown mainly on the University Campus. Plant species diversity is greater on the Campus than in parks of both cities. These woody plants are scattered over the Campus. In the central parks of both provinces, woody and herbaceous ornamental plants are grown together. *Ficus* trees are densely grown in the central parks in Adana than in Mersin. Ten *F. microcarpa* plants in each location were chosen and ten leaves with galls were collected from each plant randomly. The galls were put into the plastic bags and a total of 100 galls for each sampling location were evaluated. Samples were taken from the *Ficus* trees at 08:00-10:00 hours in July. All specimens were kept in 70-80% ethanol just after collecting. Thrips and anthocorid specimens were counted individually under a stereomicroscope (Olympus SZ51) with 45x magnifications.

**Identification of insects**

Thrips specimens were kept in the AGA (9 part 60% ethyl alcohol, 1 part glacial acetic acid, and 1 part glycerin) solution for two days and then, the specimens were taken into 5% NaOH media, and thrips specimens were kept in this media until a slight color change occurred on their bodies. The specimens were taken to the Hoyer media and slide-mounted.

Following the collection of *M. indica*, the specimens were dried and mounted for observation of their genitalia. For examination and illustration of genitalia, the specimens were macerated in 5% hot KOH solution until the organs became transparent. They were dissected with micro-pins and forceps in glycerin on a glass slide under a binocular microscope (Nikon Stereoscopic Zoom Microscope SMZ1500). Then, the samples were taken to the Hoyer media and mounted. The identification of *M. indica* was made by using the keys Carayon (1961), Yasunaga (1997), and Yamada et al (2011). Thrips specimens (*G. ficorum* and *A. ramachandrai*) were identified by the second author, and dissections of *M. indica* female and male genitalias' were done by the fourth author.

**Statistical analysis**

Eggs, nymphs, and adults of *G. ficorum* were counted under the stereomicroscope with 45x magnifications. Only adults of *A. ramachandrai* were considered in insect counting because a few numbers of larvae of the predatory thrips were noted. The nymphs and adults of the predatory hemipteran insect were counted and recorded. Seasonal total numbers of each species for each sampling location were given in Table 1. Total prey (pest thrips): predatory insect (predatory thrips or predatory bug) ratios are calculated separately for each predatory insect species and ratios were summarized

in Table 2. Generalized Linear Model (GLM) analysis was used to determine the effects of sampling location and trees on the abundance of thrips and predatory insects. According to GLM, different stages of *G. ficorum* were dependable variables. Location was a fixed factor, replicates were a random factor and predatory insects were covariates. Kolmogorov-Smirnov test and Levene's test were used to determine normality and homogeneity of variance, respectively. One-way analysis of variance (ANOVA) was conducted on mean insect numbers at different locations, followed by Turkey's post hoc mean separation. Moreover, Quadratic Regression Analyses were done to determine the relationship between mean numbers of predatory insects and mean numbers of different stages of *G. ficorum* at importance level of $P<0.05$.

Table 1. Mean and total numbers of *Gynaikothrips ficorum* and their predators on *Ficus microcarpa* per gal in three sampling locations in 2020.

| Location | *Gynaikothrips ficorum* | | | | | | *Androthrips ramachandrai* | | *Montandoniola indica* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Adults | | Nymphs | | Eggs | | | | | |
| | Mean | Total | Mean | Total | Mean | Total | Mean | Total | Mean | Total |
| University Kampüs | 10.5 ± 1.47a | 1050 | 7.62 ± 1.66a | 762 | 49.44 ± 4.11b | 4944 | 1.07 ± 0.29b | 107 | 3.16 ± 0.41a | 316 |
| Adana City Center | 6.07 ± 1.15b | 517 | 5.39 ± 0.95a | 539 | 5.70 ± 1.62c | 570 | 1.42 ± 0.23a | 142 | 0.08 ± 0.03c | 8 |
| Mersin (Seaside) | 3.92 ± 0.49b | 392 | 0.34 ± 0.17b | 34 | 63.37 ± 4.86a | 6337 | 0.00 ± 0.00c | 0 | 1.37 ± 0.23b | 137 |
| Total | - | 1959 | - | 1335 | - | 11851 | - | 249 | - | 461 |

*Means with same letter in the same column are not statistically significant according to Tukey's HSD test ($P < 0.05$).

## RESULTS

### Characterizations of pest, predatory thrips, and predatory bug

*Gynaikothrips ficorum* was reported for the first time on *Ficus* in Turkey by Toros and Tunç (1988), with descriptions in detail. This published paper and also a key provided by Priesner (1939) were used to identify the specimens of the pest thrips collected in the present study. Adult females of *G. ficorum* are black in color (Fig. 1A). Fore tibiae are yellow, tips of mid and hind tibiae are light yellow, and all tarsus is light yellow. The first and second antenna segments are dark, the rest are light yellow, the apical half of the 7$^{th}$ antenna, and the 8$^{th}$ antennal segments are in gray. The wings are transparent in color. Head length is 1.3 times its width. Postocular setae is short, does not protrude from the side of the head. Setae of the anterior corner of the prothorax vary between 40-50 microns, but epimeral setae between 135-150 microns. Metanotum and anterior abdominal segments are reticulate. The lengths of anal setae in the abdomen are 275-290 microns.

Adult males are smaller than females. The tenth abdominal segment is shorter. The forelegs are weak; the toothed protrusion in the tarsus is smaller than that of the female.

Larvae and pupae are transparent and sometimes light yellowish. Wing protrusions are evident in pupae. Antennas elongated backward (Fig. 1B).

Table 2. Relationship among numbers of *Gynaikothrips ficorum* and their predators.

| Locations | Predators | Pest stages | $R^2$ | F | P | Equations |
|---|---|---|---|---|---|---|
| Adana City Center | *Androhrips ramachandrai* | Egg | 0.7448 | 10.217 | 0.008 | $y = 14,22x^2 - 32,838x + 19,426$ |
| | | Nymph | 0.1488 | 0.612 | 0.569 | $y = 2,5489x^2 - 8,3724x + 11,381$ |
| | | Adult | 0.5277 | 3.911 | 0.072 | $y = -4,052x^2 + 12,653x - 3,4215$ |
| | *Mantondoniola indica* | Egg | 0.402 | 2.353 | 0.165 | $y = 633,75x^2 - 79,125x + 4,425$ |
| | | Nymph | 0.1216 | 0.485 | 0.635 | $y = -98,75x^2 + 9,375x + 5,825$ |
| | | Adult | 0.0394 | 0.144 | 0.869 | $y = -16,25x^2 - 1,375x + 5,475$ |
| University Campus (in Adana) | *Androhrips ramachandrai* | Egg | 0.4366 | 2.712 | 0.134 | $y = 39,254x^2 - 128,84x + 120,45$ |
| | | Nymph | 0.0147 | 0.052 | 0.950 | $y = 0,4959x^2 - 1,9436x + 8,8551$ |
| | | Adult | 0.1851 | 0.795 | 0.489 | $y = -3,0433x^2 + 6,8873x + 8,3134$ |
| | *Mantondoniola indica* | Egg | 0.0022 | 0.008 | 0.992 | $y = 1,2022x^2 - 8,1087x + 61,695$ |
| | | Nymph | 0.3521 | 1.902 | 0.219 | $y = -1,3092x^2 + 9,8097x - 8,8203$ |
| | | Adult | 0.4655 | 3.048 | 0.112 | $y = -2,477x^2 + 14,788x - 8,6847$ |
| Mersin (Seaside) | *Androhrips ramachandrai* | Egg | - | - | - | - |
| | | Nymph | - | - | - | - |
| | | Adult | - | - | - | - |
| | *Mantondoniola indica* | Egg | 0.4834 | 3.275 | 0.099 | $y = -10,527x^2 + 48,88x + 19,764$ |
| | | Nymph | 0.5245 | 2.775 | 0.034 | $y = 0,7343x^2 - 1,3816x + 0,6033$ |
| | | Adult | 0.3588 | 1.959 | 0.211 | $y = 0,1566x^2 + 1,4869x + 1,5355$ |

"-"predatory thrips was not detected.

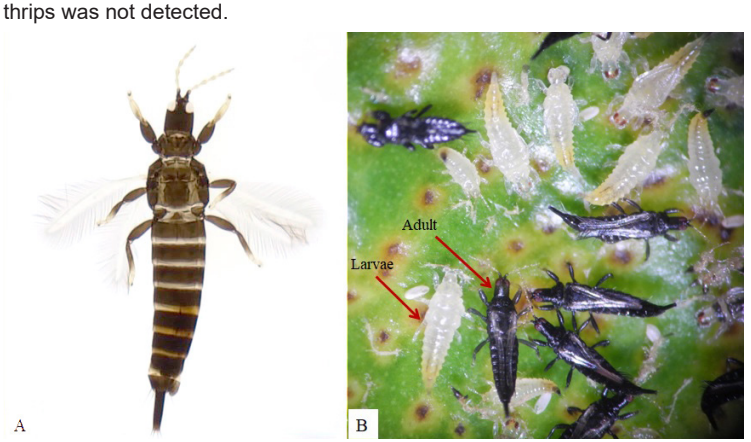


Fig. 1. Dorsal view of *Gynaikothrips ficorum*, (A) slide mounted female, (B) natural view of various biological stages.

There are about 40 species in the genus *Gynaikothrips*, the majority of which have been described in southeastern Asia. This genus is poorly defined and the species distinction is often difficult. *Gynaikothrips ficorum* and *Gynaikothrips uzeli* (Zimmerman, 1990) (Thysanoptera: Thripidae) are very similar species to each other. *Gynaikothrips ficorum* is distinguished from the *G. uzeli* species by having the pronotal posteroangular setae being longer than the discal setae (Mound et al, 1996).

The key provided by Cavalleri, Linder, & Mendonça et al (2016) was used to identify *Androthrips*. Female of the predatory thrips (*A. ramachandrai*): Body and legs brown, tarsi yellow, also fore tibiae and apices of mid and hind tibiae; antennal segment III yellow, IV-VI yellow with apex variably brown; fore wings pale. Antenna with 8 segments, segment III with 3 sensoria, IV with 4 sensoria. Pronotum with no sculpture, 4 pairs of major setae present, anteromarginals minute. Fore femur often enlarged, with rounded tubercle on inner margin near the base; fore tarsal tooth often large (Fig. 2). Male smaller; tergite IX setae S2 short and stout. The genus *Androthrips* includes 12 species, 11 of which have been described in Tropical Asia. The species are morphologically similar to each other. *A. ramachandrai* has brown tibias. In contrast, yellow tibia is found in *A. monsterae* Moulton (Mound & Minaei, 2007).

The general shape and structure of genitalia in obtained specimens from Turkey have almost the same as the type specimens of *M. indica*. The male paramere has a long and very slightly sinuate flagellum which is approximately twice as long as the cone (Fig. 3A). The female mating tube is much longer and its apex is reaching or exceeding the anterior margin of sternum VII (Fig. 3B). Copulatory tube in obtained specimen from Turkey is proximate to the base of the ovipositor; this condition a little differs from those of type specimens (Yamada et al, 2011). The difference in the copulatory tube between Turkish specimens and Indian specimens might be a geographic variation or individual variation. The type specimens have been compared with by the last author; it concluded that these species agree with *M. indica*. Type specimens are now deposited in the Tokushima Prefectural Museum where the last author is.

**Total numbers of insect species identified**

With this study, totally 249 adults of *A. ramachandrai* and 461 individualls of *M. indica* were detected in the galls infested with *G. ficorum*. Moreover, 1959 adults, 1335 immatures and 11851 eggs of *G. ficorum* were also recorded. Galled leaves collected from trees in Çukurova University Campus had the highest number of prey thrips and predatory insect species. Relatively high number of predatory thrips was recorded on galled leaves collected from *F. microcarpa* in Adana city center. In Mersin, no predatory adult thrips was found in the galls, but hemipteran predators were determined (Table 1).

**Prey: predator interactions**

According to GLM analysis, location is the most important factor that can affect the abundance of pest thrips and predatory insect species. Moreover, there was a positive interaction between pest eggs-*A. ramachandrai* adults in Adana City Center and pest nymph-*M. indica* adults in Mersin (Seaside) (Table 2). While no *A. ramachandrai* were

found in the sampling area in Mersin, the egg numbers of harmful thrips was found to be higher than in the other sampling area. Similarly, in Adana City Center, predator thrips was at the highest level, while the harmful thrips eggs remained at the lowest level.

Fig. 2. Dorsal view of slide mounted female of *Androthrips ramachandrai*.

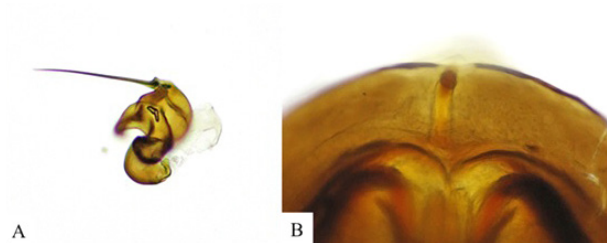


Fig. 3. Male paramere (A) and female copulatory tube (B) of *Montandoniola indica*.

In the current study, the ratios of the thrips per predatory species are shown in Table 3. Since *A. ramachandrai* adults were determined mostly in Adana City Center, the rates of prey per predator were the lowest. Since *M. indica* was detected extremely low in the Adana city center, the ratios of the *G. ficorum* per predator were the highest. *Montandoniola indica* was found with lower rates in the other survey areas. The ratios of mobile stages of *G. ficorum* per predators' *A. ramachandrai* and *M. indica* on galled leaves of *F. microcarpa* in the University Campus were under the 1:10 and 1:5 respectively. In the Adana City Center, these ratios were around 1:5 and 1:71 per predator. And also, the ratios of mobile stages of *G. ficorum* per *M. indica* were under 1:3 in Mersin (Seaside).

Table 3. The ratios of *Gynaikothrips ficorum* per predators (*A. ramachandrai* and *M. indica*) on galled leaves of *F. microcarpa* in three sampling locations in 2020.

| Locations | *Androthrips ramachandrai* | | | *Montandoniola indica* | | |
|---|---|---|---|---|---|---|
| | Prey* adults | Prey larvae | Prey eggs | Prey Adults | Prey Larvae | Prey Eggs |
| University Kampüs | 9.81 | 7.12 | 46.21 | 3.32 | 2.41 | 15.65 |
| Adana City Center | 3.64 | 3.80 | 4.01 | 64.63 | 67.38 | 71.25 |
| Mersin (Seaside) | - | - | - | 2.86 | 0.25 | 46.26 |

*Prey= *Gynaikothrips ficorum*; "-"predatory thrips was not detected.

## DISCUSSION

In The Cuban laurel thrips has been widely distributed with the ornamental plant marketing of decorative *Ficus* spp. throughout all continents, except Antarctica (Mound, 2009). This thrips represents a microcosm and hosts all stages of pest thrips and their natural enemies, such as *A. ramachandrai* and *Montandoniola* spp.. The strong associations were determined with the populations of gall-forming thrips, *G. ficorum*, *G. uzeli* and their natural enemies (Paine, 1992; Boyd & Held, 2006; Cambero-Campos et al, 2010; Arthurs et al, 2011; Yamada et al, 2011; Tavares et al, 2013). Following the trade of the *Ficus* trees and an increase in the number of the host plants of *G. ficorum*, this pest thrips has been widely distributed to the region recently and also cause damage to *F. microcarpa* in outdoor landscapes ( & Pehlivan, 2019). Although many studies reported the interactions of the natural enemies of *G. ficorum*, until this time there is no record from Turkey. With the current study, *A. ramachandrai* and *M. indica* were reported for the first time in Turkey in 2020. *Gynaikothrips ficorum* and its natural enemies can be introduced with the horticultural trade of *Ficus* trees in Turkey (Atakan & Pehlivan, 2019). Çukurova University Campus had the highest number of prey thrips and predatory insect species. In University Campus representing rich plant and insects' biodiversity, where no insecticide application against the pest thrips on the ficus trees was performed. Therefore we assumed that *G. ficorum* and its natural enemies were more abundant in comparison to other habitats surveyed.

With the current study, there was a positive relationship between the mean numbers of *G. ficorum* eggs and *A. ramachandrai* adults in Adana City Center. Although the Metropolitan Municipality has applied insecticide to control *G. ficorum*, the predator thrips numbers were the highest. There may be different reasons for the presence of many predatory thrips in the Central park of Adana. At least, the high number of Ficus trees in this sampling unit may have positively affected the population development of predatory thrips as well as the harmful thrips. Genus *Androthrips* includes 12 species (Mound, 2013) and is often considered to be predators on the other gall-forming thrips species in Asia (Varadarasan & Ananthakrishnan, 1981; Sureshkumar & Ananthakrishnan, 1987). *Androthrips ramachandrai* was described from India and found in associate with galls formed by the *Austrothrips cochinchinensis* Karny (Thysanoptera: Phlaeothripidae) on *Calycopteris floribunda* (Roxb.) Lam. (Combretaceae) (Anantakrishnan, 1978). Moreover, this predatory thrips has been reported as an important predator of *Gynaikothrips*, and many researchers have observed *A. ramachandrai* feeding on eggs, larvae, and pupae of *G. uzeli* and *G. ficorum* in galls of *Ficus* spp. (Boyd & Held, 2006; Held & Boyd, 2008; Cavalleri et al 2011; de Melo, Cavalleri, & Mendonça, 2019).

In this study, *M. indica* was widely distributed in the sampling areas with its prey *G. ficorum*. There was a positive interaction between the numbers of pest nymphs and *M. indica* adults in Mersin (Seaside). And also, predator:prey ratios among the mobile stages of the pest thrips and the predatory bug were less than 1:4 in University Campus and Mersin (Seaside) indicating that harmful thrips was at higher risk of predation due to the *M. indica.* The relatively higher numbers of predatory bug in

parks in Mersin compared to that of Adana may be related to presence of different ecological factors, such as climate, plant diversity, and whether there is an insecticide application or not.. *Montandoniola* spp. are the predators of family Phlaeothripidae (Insecta: Thysanoptera) including five species belonging to the genus *Gynaikothrips* (Dobbs & Boyd, 2006; Yamada et al, 2011). An anthocorid described as *M. moraguesi* (before taxonomic revision) has been intentionally released for classical biological control of *G. ficorum* from the Philippines to Hawaii and Bermuda, achieved long-term control on outdoor plantings of ornamental *Ficus* spp. (Davis & Krauss, 1966; Funasaki, 1966; Cock, 1985). Despite the availability of different life stages of *G. ficorum* in the gall, *M. confusa* adults preferred to prey upon on thrips eggs, with an estimated 10-fold greater predation on eggs compared to other stages of thrips (Tavares et al 2013). Besides, Arthurs et al (2011) showed that *M. confusa* reproduced throughout the year and reduced *G. uzeli* population's ≥95% and leaf galls by up to 77% within 5 weeks on three *F. benjamina* cultivars in greenhouses. Recently, Yamada et al (2011) examined the Indian specimens of *Montandoniola* and identified *M. indica* as a new species which was associated with the gall-forming thrips, *Liothrips karnyi* Bagnall, 1924 (Thysanoptera: Phlaeothripidae), on black-pepper, *Piper nigrum* Linnaeus (Piperaceae), in southern India. Recently, Ali & Streito (2019) have been detected two heteropterous species *M. indica* and *Geocoris amabilis* Stål, 1855 (Heteroptera: Geocoridae) on the leaves of *Ficus benjamina* which was infested with *G. uzeli*, in the coastal area in Tartous, Syria. This was the first detection of *M. indica* outside India. Tartous province is only 400 km far from Adana province, Turkey. Although there has been limited information about the distribution area of *M. indica*, the predatory bugs are potentially much wider than the only localities reported by Yamada et al (2011). It is not possible to know whether it originates from Turkey, Syria, or is introduced. Moreover, Yamada et al (2011) indicated that 1$^{st}$ instar of the predatory bug preferred eggs and larvae of thrips, 2$^{nd}$ instar nymphs fed on thrips larvae as well, whereas older nymphal stages and adults of *M. indica* fed mainly on adult thrips. Ballal, Gupta, & Sunil (2012) reported that *M. indica* can be successfully reared on the rice moth, *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae) eggs, and also the mass-produced predator can be evaluated as a biological control agent of *L. karnyi* infesting black pepper in India.

In this study, the ratios of different biological stages of *G. ficorum* per the predators'; *A. ramachandrai* and *M. indica*, on galled leaves of *F. microcarpa* were ranged between 1:3 and 1:71 at different surveying locations. There was no study related to the predator: prey interactions among this species. However, Sabelis & van Rjin (1997) reported that a predator: prey ratio of 1:217 may be sufficient in the long term to control thrips by anthocorid predators, while thrips can be suppressed in a shorter time at a ratio of 1:51. Similarly, Funderburk, Stavisky, & Olson (2000) also determined that the 1:40 ratio was sufficient for *O. insidiosus* to control the *F. occidentalis* population in pepper fields in a short time. In the light of these studies, the predators might be an important factor to control *G. ficorum* on *Ficus* trees in Adana and Mersin Provinces.

In conclusion, this study deals with the first records of *A. ramachandrai* and *M. indica* in galled leaves infested by the *G. ficorum*. However, with the limited number of samplings in the locations, these results indicate that both predatory insect species may play a crucial role in suppressing the pest thrips on *F. microcarpa* growing in natural habitats such as the University Campus. This situation is more important in terms of the protection of the environment and human health. The control of this harmful thrips species on Ficus trees grown in the urban landscapes by using pesticides could be difficult due to its cryptic and feeding behaviors, and pesticides may also pose more risks to the environment and human health. Therefore, the role of natural enemies to achieve long-term control of this pest should be investigated with the laboratory and field experiments such as mass releases of the predatory insects detected in the region. Moreover, the population dynamics and the interactions of the pest thrips and its predators in different habitats hosting Ficus trees should be studied in a more detail in the following years.

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*J. Entomol. Res. Soc., 24(3): 281-290, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2177* *Online ISSN:2651-3579*

# New Record of *Pentalonia nigronervosa* Coquerel, 1859 (Hemiptera, Aphididae) in Turkey

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## ABSTRACT

The banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), is a major pest of banana, *Musa* spp., (Zingiberales) has worldwide distribution. This study was conducted to detect aphid species in the banana production regions of Adana, Antalya, and Mersin in Turkey. Aphid samples have been collected in total of thirty-seven locations. All the samples were identified as *P. nigronervosa.* Later on, molecular characterization has been done based on COI and it was recognized that the Turkish population was close to Florida and some Hawaiian genotypes. *Pentalonia nigronervosa* was recorded for the first time in Turkey in this study. Sustainable nature-friendly management tactics for controlling *P. nigronervosa* which poses less health threat to human should be developed.

*Key words*: Banana aphid, *Musa* spp, *COI*, mtDNA, Turkey, vector.

Yayla, M. (2022). New record of *Pentalonia nigronervosa* Coquerel, 1859 (Hemiptera, Aphididae) in Turkey. *Journal of the Entomological Research Society*, 24(3), 281-290.
*Received: November 16, 2021* *Accepted: July 26, 2022*

## INTRODUCTION

The banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), is one of the most important pests of banana (Mathers, Mugford, Hogenhout, & Tripathi 2020). The pest was also recorded on *Heliconia* spp., *Caladium* spp., *Alpinia* spp., and *Dieffenbachia* spp., *Colocasia esculenta* (Blackman & Eastop, 1984; Waterhouse, 1987). The banana aphid was described on the island of Madagascar in the 19th century (Coquerel, 1859). *Pentalonia nigronervosa* causes damage by sucking on the phloem of banana plants grown in tropical and subtropical areas and greenhouses in the world (Blackman & Eastop, 1984; Robson, Wright, & Almeida, 2007). High aphid population density may suppress the growth of young plants or cause die back of the plants, whereas direct damage to mature plants is often negligible (Mathers et al, 2020).

*Pentalonia nigronervosa* feeds on the stem, late leaves, and tips of the growth point of the banana, and produces honeydew causing fumagin over time (Waterhouse & Norris, 1987). The pest also forms big colonies on 7-8 cm below the soil surface in colonies are feeding leaf sheaths of the banana plant and the stem. Feeding damage of *P. nigronervosa* on the banana plant (*Musa* spp.) causes the formation of stunting, spotting on the fruit, fumagin, as well as transmission of Banana Bunchy Top Virus (BBTV) as a vector (Drew, Moisander, & Smith, 1989).

*Pentalonia nigronervosa* usually has a viviparous life cycle (Bhanotar & Ghosh, 1969); it goes through four nymph periods before it becomes mature. The pest, which can reproduce 30 generations in a year under favorable conditions, has a high reproductive capacity. The nymph development stage was detected as 9-15 days, whereas longevity is lasting between 9.9 and 12.5 days, and a female gives 3-20 offspring under laboratory conditions (Rajan, 1981; Viswanathan & Regupathy, 1992). Lomerio & Calilung (1993) reported the life cycle of *P. nigronervosa* on a few hosts in greenhouses as 6-21 to 20-28 days. Moreover, the pest has annually four generations in Guangdong (China), and the population density is high in April and September - October (Yang, 1989). Alatea forms migrate from plant to plant within the same orchards or to different locations in the ecological area and reach high populations in the growing plant (Waterhouse & Norris, 1987).

Due to the agricultural subventions and high profits, the production of bananas under the greenhouse has started to increase rapidly in Adana, Mersin, and Alanya provinces in the Mediterranean region, The banana production and its profit reaching Anamur's level, the production area where bananas have been grown for a long time. While the production of domestic bananas in Turkey was around 50 thousand tons in 2000, it has now increased to 728 thousand tons (TUİK, 2020). Increasing banana production brought plant protection problems. Seedlings of new varites may imported to meet the increasing demand in production areas, prepare the basis for bringing new pests and diseases.

Here it is reported that *Pentalonia nigronervosa*, a native species of tropical rainforest climate zones, was diagnosed for the first time by morphological and molecular methods on banana plants grown area in Turkey. This report includes new information on the host range and distribution of the species in Turkey.

## MATERIAL AND METHOD

### Sample collection

Wingled and wingless adult aphids were collected from commercial banana production areas which are coastal Mediterranean regions from Adana (n:2), Hatay (n:3), and Mersin (n:32) provinces in Turkey during spring and autumn in 2020 (Table 1). The aphids were collected from infested banana plants with a small brush and preserved in 70 percent ethyl alcohol in the Epphendorph tubes and taken to the Republic of Turkey Ministry of Agriculture and Forestry Biological Control Research Institute for examination.

Table 1. The list of banana aphid specimens examined in the study from Adana, Mersin, and Hatay provinces of Türkiye, including collection date, location, coordinate, size of the field or greenhouse, and variety.

| No | Location | Date | Size (decare) | Variety | Coordinate | |
|---|---|---|---|---|---|---|
| | | | | | Latitude (E) | Longitude (N) |
| 1* | Erdemli /Mersin | 18.03.2020 | 6 | Grand Naine | 36°33’59’’ | 34°14’08’’ |
| 2 | Arsuz/ Hatay | 12.06.2020 | 22 | Grand Naine | 36°27’’06’’ | 35°57’43’’ |
| 3 | Anamur/ Mersin | 03.04.2020 | 23 | Şimşek | 36°03’25’’ | 32°48’57’’ |
| 4 | Anamur/ Mersin | 03.04.2020 | 24 | Grand Naine | 36°03’25’’ | 32°48’57’’ |
| 5 | Anamur/ Mersin | 03.04.2020 | 25 | Şimşek | 36°03’25’’ | 32°48’57’’ |
| 6 | Anamur/ Mersin | 03.04.2020 | 26 | Grand Naine | 36°03’26’’ | 32°48’59’’ |
| 7 | Anamur/ Mersin | 03.04.2020 | 27 | Grand Naine | 36°03’26’’ | 32°48’59’’ |
| 8 | Anamur/ Mersin | 03.04.2020 | 28 | Bodur Azman | 36°03’42’’ | 32°48’49’’ |
| 9 | Anamur/ Mersin | 03.04.2020 | 29 | Bodur Azman | 36°03’42’’ | 32°48’49’’ |
| 10 | Anamur/ Mersin | 21.04.2020 | 30 | Şimşek | 36°03’19’’ | 32°47’37’’ |
| 11 | Anamur/ Mersin | 21.04.2020 | 38 | Azman | 36°03’42’’ | 32°50’36’’ |
| 12 | Anamur/ Mersin | 21.04.2020 | 39 | Bodur Azman | 36°02’37’’ | 32°49’28’’ |
| 13 | Bozyazı/ Mersin | 21.04.2020 | 40 | Azman | 36°07’20’’ | 32°57’48’’ |
| 14 | Bozyazı/ Mersin | 21.04.2020 | 41 | Azman | 36°07’19’’ | 32°57’38’’ |
| 15 | Bozyazı/ Mersin | 21.04.2020 | 42 | Azman | 36°07’19’’ | 32°57’50’’ |
| 16 | Bozyazı/ Mersin | 21.04.2020 | 43 | Azman | 36°07’16’’ | 32°57’45’’ |
| 17* | Bozyazı/ Mersin | 06.05.2020 | 44 | Azman | 36°07’13’’ | 32°57’47’’ |
| 18* | Bozyazı/ Mersin | 06.05.2020 | 45 | Grand Naine | 36°07’00’’ | 32°57’14’’ |
| 19 | Bozyazı/ Mersin | 06.05.2020 | 46 | Şimşek | 36°07’01’’ | 32°57’16’’ |
| 20 | Bozyazı/ Mersin | 06.05.2020 | 47 | Bodur Azman | 36°07’12’’ | 32°56’59’’ |
| 21* | Bozyazı/ Mersin | 06.05.2020 | 53 | Grand Naine | 36°07’53’’ | 32°56’54’’ |
| 22 | Bozyazı/ Mersin | 06.05.2020 | 54 | Bodur Azman | 36°06’57’’ | 32°57’41’’ |

Table 1. Contınued.

| No | Location | Date | Size (decare) | Variety | Coordinate | |
|---|---|---|---|---|---|---|
| | | | | | Latitude (E) | Longitude (N) |
| 23 | Bozyazı/ Mersin | 06.05.2020 | 55 | Grand Naine | 36°06'57" | 32°57'40" |
| 24 | Bozyazı/ Mersin | 06.05.2020 | 56 | Azman | 36°06'57" | 32°57'39" |
| 25 | Bozyazı/ Mersin | 06.05.2020 | 57 | Şimşek | 36°06'58" | 32°57'39" |
| 26 | Bozyazı/ Mersin | 06.05.2020 | 58 | Şimşek | 36°06'45" | 32°57'14" |
| 27 | Bozyazı/ Mersin | 06.05.2020 | 59 | Bodur Azman | 36°06'15" | 32°56'54" |
| 28* | Arsuz/ Hatay | 18.05.2020 | 60 | Azman | 36°32.0400 | 36°6.1930 |
| 29 | Arsuz/ Hatay | 18.05.2020 | 25 | No Data | 36°26.7810 | 35°57.9100 |
| 30* | Yumurtalık/Adana | 05.06.2020 | 30 | No Data | 36°27.1020 | 35°57.7130 |
| 31 | Yumurtalık/Adana | 05.06.2020 | 25 | No Data | 36°56.2200 | 35°4.8680 |
| 32 | Silifke/ Mersin | 18.03.2020 | 20 | No Data | 36°20.8590 | 34°14'55" |
| 33* | Taşucu/ Mersin | 18.03.2020 | 20 | No Data | 36°20.7650 | 34°15'00" |
| 34 | Silifke/ Mersin | 18.03.2020 | | Grand Naine | 36°22'28" | 34°01'53" |
| 35 | Silifke/ Mersin | 18.03.2020 | 50 | Grand Naine | 36°22'08" | 34°03'15" |
| 36 | Silifke/ Mersin | 18.03.2020 | 150 | Grand Naine | 36°20'43" | 34°03'03" |
| 37 | Silifke/ Mersin | 18.03.2020 | 10 | Grand Naine | 36°22'17" | 34°01'48" |

*the sample used for molecular studies.

## Morphological identification

The Slide mounting technique was applied to wingled and wingless adut aphids mainly based on the method of Hille Ris Lambers, (1950). The specimens were studied using a LEICA DM LB2 compound light microscope for identification. The species determination by an expert was done using Heie (1995), Blackman & Eastop (1994, 2000, 2006, 2020). The specimen vouchers are preserved in Nazife Tuatay Plant Protection and Insect Museum in Ankara.

## Molecular identification

## DNA isolation and PCR reaction

The genomic DNA of *P. nigronervosa* collected from nine locations (n = 18) was isolated according to DNAeasy Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's recommendations. DNA quality and quantity were measured with a microplate reader (Multiscan GO, Thermo) and kept at -20 °C until PCR reaction. LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HC02198 (TAAACTTCAGGGTGA CCAAAAAATCA) primer pairs used for PCR applications (Lourenço et al, 2006). PCR reaction was prepared as 50 µl final volumes with 5 µl Taq buffer (10X), 2,5 mM $MgCl_2$, 250 µM dNTPs, 1 µM primer, 0,5 U Taq, 1 µl DNA.

Thermocycler (Applied Biosystems (Veriti)) condition was adjusted to 5 min. at 94°C for pre-denaturation, 1 min. at 94°C for denaturation, 1 min. at 50°C for annealing, 1 min. at 72°C for the extension as 35 cycles and 7 min. at 72°C for the last extension. The PCR reactions were verified using Agarose (1%) gel and gel imagen system. The Sanger sequencing was applied to the PCR products by Molgentek®, Istanbul, Turkey.

### Bioinformatic analyses

The sequences of *P. nigronervosa* were verified by comparing with other reference genes in NCBI (The National Center for Biotechnology Information (http://www.ncbi.nlm.gov/BLAST)) Gene bank. The sequences translated to Fasta format were aligned at the Mega 7 program (Kumar, Stecher, & Tamura, 2016). The correction of sequences was done on the Finch TV program. The maximum likelihood Method with 2000 boostrap value was used for the phylogenetic relationship between them. *Aphis gossypii* Glover was selected as the outgroup.

## RESULTS

### Description

*Pentalonia nigronervosa* is small, red-brown to almost black in the nymphal period, and completely black during adulthood. The height of the adults varies between 1.1-1.8 mm. Their bodies are oval to cylindrical and slightly swollen. The species has a pair of corniculus on both sides of the body towards the end of the abdomen. It has both winged and wingless forms. The body of wingless viviparous females is 1.1 to 1.8 mm in length, the head is pale or black. The first segment of the antennas (Scapus), the length of the body, is black and the remaining part is pale. The corniculus is black-pale colored. Legs are black-pale band-shaped. In winged viviparous females, the body is 1.1-1.8 mm in length, the head and thorax are black and the abdomen is pale-black. The eyes are blackish. The wing veins are broadly lined with brown pigments that accentuate the unusual venation. (Blackman & Eastop, 1984) (Figs. 1, 2).

### Distribution

The species is widespread in all tropical and subtropical parts of the world (EUROPE, Azores, ASIA, Bonin Islands, Ceylon, China, Christmas Island, Cocos-Keeling, India, Indonesia, Israel, Jordan, Malaysia, Nepal, Bangladesh, Philippine Islands, Ryukyu Islands, Taiwan, AFRICA, Angola, Burundi, Cameroun, Canary Islands, Cano, Verde Islands, Congo, Dahomey, Egypt, Gambia, Ghana, Guinea, Ivory Coast, Kenya, Madagascar, Madeira, Malawi, Mali, Mauritania, Mauritius, Niger, Nigeria, Portuguese, Guinea, Réunion, Rhodesia, Rwanda, St. Helena, Senegal, Sierra Leone, Somalia, South Africa, Tanzania, Togo, Uganda, Upper Volta, AUSTRALASIA and PACIFIC ISLANDS, Australia, Caroline Islands, Cook Islands, Fiji, Gilbert and Ellice Islands, Hawaii, Mariana Islands, Marshall Islands, New Hebrides, Papua & New Guinea, Samoa, Tokelau, Tonga, Wallis, Irian Jaya, NORTH AMERICA, Mexico, U.S.A., CENTRAL AMERICA and WEST INDIES, Costa Rica, Guatemala, Honduras,

Nicaragua, Panama Canal Zone, West Indies, SOUTH AMERICA, Brazil, Colombia, French Guiana, Guyana, Surinam, Venezuela.) (CABI, 2021).


Fig. 1. Wingless (a) and winged (b) *Pentalonia nigronervosa* in Mersin, Turkey.


Fig. 2. Damage of *Pentalonia nigronervosa* on the banana plants (a, b: damage on banana shoots, c: backward death, d: severely damage).

## Molecular identification

The research was carried out to reveal aphid species from banana greenhouses in the Mediterranean Region of Turkey. For this purpose, 18 specimens from ten aphid populations were analyzed by molecular method, and 700-bp product COI regions were sequenced. *Aphis gossypii* as an out-group was separated from the other specimens. The reference genes were compared to have an idea about the origin of *P. nigronervosa* in Turkey.

According to molecular studies, aphid specimens from Turkish banana plantations were identified as *P. nigronervosa.* The research is the first record of *P. nigronervosa* in Turkey. *Pentalonia nigronervosa* specimens in the phylogenetic tree were separated into three groups. Indian specimens were identical with 96 Bootstrap values and distinguished from the others. The other cluster had Turkish specimens and was distinguished with a 97 Bootstrap value. Turkish specimens are close to Florida and

some Hawaii specimens. The last group which has Kenya and some Hawaii specimens are separated from the other two with lower Bootstrap values.

## DISCUSSION

Climatic conditions are the key factor for plant distribution. because of anthropologic activities climate-changing is observed all over the world although it has adverse effects on plants and animals in some cases. Opposite this, it provides new opportunities for cultured plants like the banana, which is a tropical plant. It has been brought from Egypt and cultured in Turkey since the beginning of the 1900s. Until the last two decades, its distribution was limited, but now it has dispersed to the entire Mediterranean region of Turkey. So Changing climatic conditions and accordingly changing plant patterns create favorable conditions for new plant and insect species to settle in a region. Banana aphid, *P. nigronervosa*, has a widespread distribution in tropic and subtropics regions of the world with more than 120 locations (CABI, 2021). Until the last 10 years, banana production was limited in Anamur district of Mersin and some districts of Antalya which have a microclimatic conditions to grow bananas in Turkey. Increasing temperatures, decreasing number of frosty nights, and subventions for production in greenhouse resulted in the spreading of banana production throughout the coast of the Mediterranean Region of Turkey with exponential growth. *Pentalonia nigronervosa* possibly entered Turkey as a result of this uncontrolled growth. Banana production regions have been exposed to *P. nigronervosa* probably due to the import of propagation material or materials imported for breeding programs and spread to all banana production areas. Though *Aphis gossypii, Aphis fabae*, and *Myzus persicae* were listed as an aphid on bananas (Uygun, Ulusoy, Karaca, & Satar, 2010), *Pentalonia nigronervosa* was found to be the only common aphid species on bananas in the entire region. The use of the conventional growing method for settling a new banana plantation causes contamination of the pest. Contaminated banana seedlings have growing problems and result in quality and quantity problems (Fig. 2). Moreover, our observation shows that the stem apex and flowering organs of mature plants encounter similar problems. Therefore, the use of material from tissue culture is an important method for preventing the spreading of the pest in controlled greenhouse conditions.

*Pentalonia nigronervosa* was first described in Isle de Bourbon of Madagascar and now had worldwide distribution in all banana-growing areas (Robson et al, 2007). Molecular studies revealed that *P. nigronervosa* clustered three different branches and one has all Turkish specimens in the world (Fig. 3). Turkish specimens have one haplotype which indicates that the aphid probably spread from one resource. Anholocyclic development of the aphid in the region contributed to fast-spreading and having one haplotype. However, the research of Galambao, (2011) in Hawaii islands refused that parthenogenetic production in aphids caused population-based differentiation. The researcher detected genetic differences not only among islands, and different host plants but also in banana populations that have more than ten genotypes. *Pentalonia nigronervosa* was listed on spinach in Hawaii by Holdaway,

(1944). Long-term adaptation of *P. nigronervosa* in Hawaii islands could be the reason for genetically differentiated populations. However, the species was first recorded with this study and possibly had only several months period in Turkey.

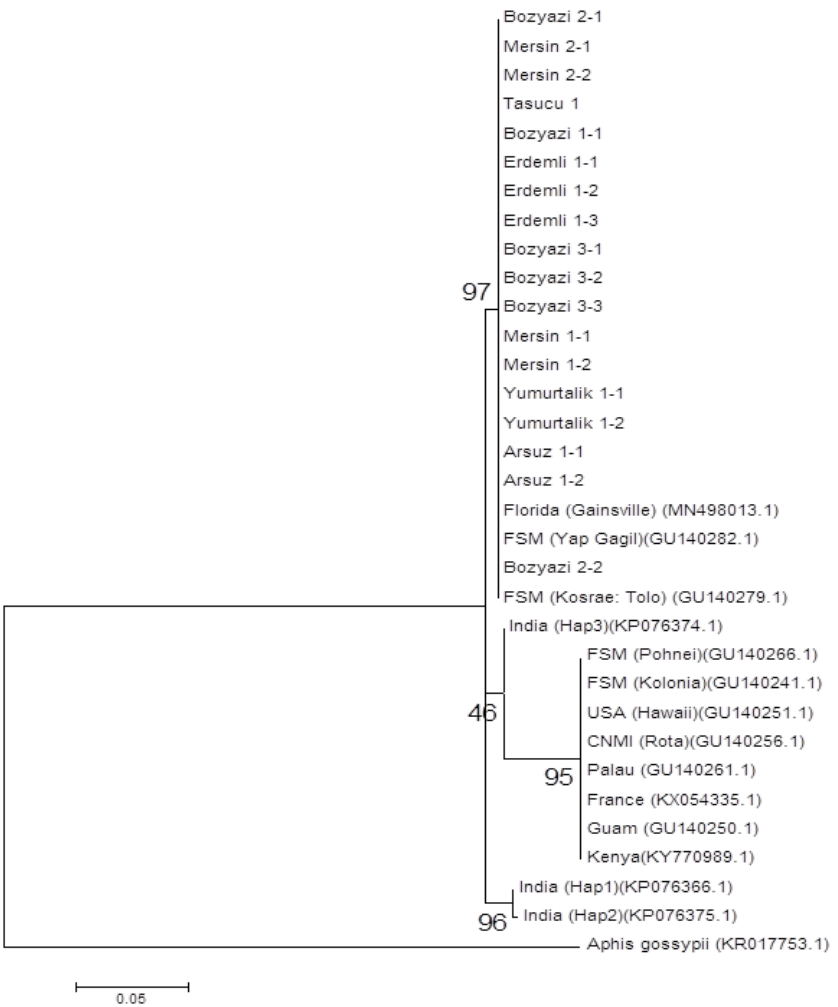


Fig. 3. Dendrogram constructed by Maximum-Likelihood method from the COI sequences of *Pentalonia nigronervosa* .

Although, all the collected samples from the different locations were cultured in the laboratory condition no biological control agents detected. *Aphidius colemani* Viereck and *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae) have the potential to control *P. nigronervosa* (Völkl et al, 1990), and these species were active in the region (Satar et al, 2014; 2021). But none of them recorded in *P. nigronervosa* in the region.

The study is the first record for the determination of *Pentalonia nigronervosa* in Turkey. During the survey period of the study, any biological control agents from cultured samples in the laboratory condition were not determined in the region. Meanwhile, no product for pest control has not been registered for banana plantations yet. *Colocasia esculenta* being planted in Bozyazı (Mersin), known as taro, is also a minor host for this aphid (Suparman, Gunawan, Pujiastuti, & Cameron, 2017; Şahin & Erbilen, 2019). While applying control practices for the banana aphid, alternative hosts should not be ignored. Biological, chemical, and cultural control methods should be integrated under the IPM strategy and applied without harming the natural balance.

**Acknowledges**

Thank you to Işıl Özdemir for morphological identification of the aphids, and Gül Satar for her contribution to molecular studies. The author also wishes to thank the General Directorate Of Agricultural Research And Policies for the financial support of the research (Project code: TAGEM/5161-GR2624).

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*J. Entomol. Res. Soc., 24(3): 291-296, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2192* *Online SN:2651-3579*

# A New Species of *Subancistrocerus* de Saussure, 1855 (Hymenoptera: Vespidae, Eumeninae) from Pakistan

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## ABSTRACT

A new species, *Subancistrocerus pakistanensis* Qasim, Carpenter et Rafique sp. nov. is described and illustrated from Pakistan, based on several specimens collected in the Multan Region. Genus *Subancistrocerus* is recorded for the first time from Pakistan.

*Key words*: Potter wasps, new species, distribution, Punjab, Vespoidea.

Qasim M., Carpenter, J. M., Khan, M. R., Rafi M. A., Hassan, M. A., Siddiqui, J. A., Hafeez, M. (2022). A new species of *Subancistrocerus* de Saussure 1855 (Hymenoptera: Vespidae, Eumeninae) from Pakistan. *Journal of the Entomological Research Society*, 24(3), 291-296.
*Received: December 02, 2021* *Accepted: November 15, 2022*

## INTRODUCTION

The genus *Subancistrocerus* de Saussure 1855, was described as a name for division I of the subgenus *Ancistrocerus* Wesmael of the genus *Odynerus* Latreille in de Saussure, 1852. It was declared available from the date of publication by Opinion 893 (ICZN, 1970). The type species, *Odynerus sichelii* de Saussure, 1855, was subsequently designated by Bequaert (1925). The genus is mainly distributed in the Oriental Region, which contains 25 species and two additional subspecies (Giordani Soika, 1994; Gusenleitner, 2000; Kumar, 2013; Li and Chen, 2014). Five species and one additional subspecies are recorded from Ethiopian Region (Carpenter et al, 2010) and two species from the Australian Region (Giordani Soika, 1993, 1994). In the present study this genus is recorded for the first time from Pakistan.

## MATERIALS AND METHODS

Wasps were collected from different localities of the Multan region during 2014-2016 by using the aerial net. Specimens were identified to the species level using Olympus SZX7, Model SZ2-ILST stereo-microscope and photographed by using Olympus SZX7 stereomicroscope attached with a Sony CCD digital camera. After the identification, all specimens were housed at National Insect Museum (NIM), National Agriculture Research Centre, (NARC), Islamabad, Pakistan, and American Museum of Natural History (AMNH), New York, USA.

### Taxonomy

#### *Subancistrocerus* de Saussure, 1855

*Subancistrocerus* de Saussure, 1855: 206. Type species: *Odynerus sichelii* de Saussure, 1855, by subsequent designation of Bequaert, 1925: 61, confirmed by Opinion 893.

*Epancistrocerus* de Saussure, 1856: 352, in Errata, substitute name for *Subancistrocerus* de Saussure. Type species: *Odynerus sichelii* de Saussure, 1855, by subsequent designation of Bequaert, 1925: 61.

Diagnostic characters. This genus is similar to some species of *Pseudonortonia* Giordani Soika, with which it shares metasomal tergum 1 with two transverse carinae. However, *Subancistrocerus* differs from *Pseudonortonia* in having tergum 1 wider than long in dorsal view and both transverse carinae close to each other at the crest of the declivity (Carpenter et al., 2009).

#### *Subancistrocerus pakistanensis* Qasim, Carpenter et Rafique sp. nov. (Figs. 1A-E)

Type material. Holotype: PAKISTAN: ♀; Punjab Province, Multan; 30.1974° N, 71.4743° E, Elev.: 141 m, August 04, 2015; leg. M. Qasim; deposited in NIM, NARC, Islamabad, Pakistan.

Paratypes. PAKISTAN: ♀; Same data as holotype; deposited in NIM, NARC, Islamabad, Pakistan and AMNH, New York, USA.

Etymology. The specific name *pakistanensis* refers to the country name “Pakistan” where the type specimens were collected.

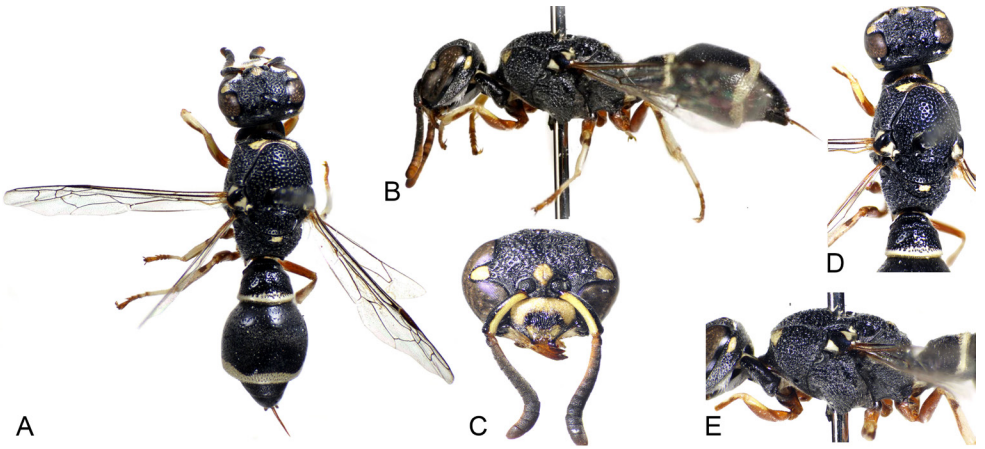


Fig. 1. A. *Subancistrocerus pakistanensis* Qasim, Carpenter et Rafique, sp. nov. Female (holotype), dorsal habitus; B. Same, lateral view; C. Head, frontal view; D. Mesosoma, dorsal view; E. Same, lateral view.

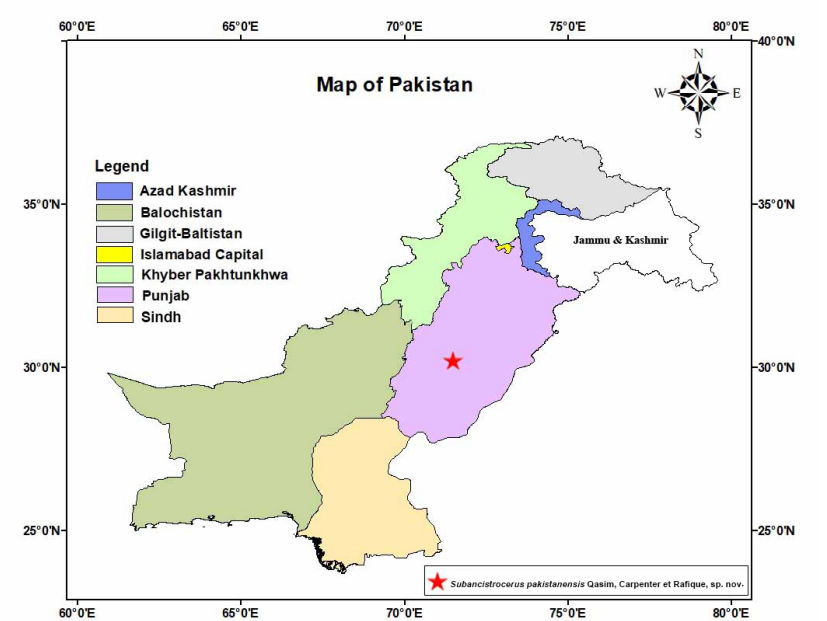


Fig. 2. Distribution map of *Subancistrocerus pakistanensis* Qasim, Carpenter et Rafique, sp. nov.

**Diagnostic characters and remarks**

Distinguished from other species of *Subancistrocerus* by the combination of having the first metasomal segment narrow and short and the body black with abundant whitish yellow markings. According to Giordani Soika (1994), key this species out to *S. sichelii* (de Saussure), the type of the genus, which has been recorded from India. Like that species, the pale markings are whitish yellow. But the pale markings are much less abundant in *S. sichelii* than in this species, with the tegula being entirely black in all the specimens at the American Museum of Natural History (AMNH) while the tegula is whitish except medial stripe black in this species. The first metasomal segment is differently shaped in the two species, being narrower and shorter in the *S. pakistanensis* specimens, which are also smaller in size. The shape is like that of *S. venkataramani* Kumar, but the pale markings in that species are bright yellow.

Description. *Holotype female.*

Body length approximately: head 0.53 mm, mesosoma 2.81 mm, T1+T2 3.03 mm; forewing length approximately: 5.21 mm.

Structure. Clypeus (Fig. 1C) truncate apically, sparsely punctate and depressed medially. Labrum (Fig. 1C) short. Mandible five-dentate with long distal tooth. Interantennal space with median frontal prominence (Fig. 1C). Area between eyes and antennae without macropunctures. Frons, vertex and temple with coarse punctures (Figs 1C-D). Vertex short. Cephalic foveae small and contiguous. Pronotum, scutum, scutellum, propodeum and mesopleuron densely punctate except metapleuron largely impunctate (Figs 1D-E); punctures larger and deeper than those on head; anterior face of pronotum with median foveae surrounded with smooth area; propleuron with fine, dense punctures. Tegula campanulate. Parategula from its hind margin strongly concave. Propodeum shallowly coarsely punctate, with submarginal carina projecting as long tooth (Fig. 1D). Tergum 1 (T1) with two transverse carinae (Fig. 1D). Sternum 1 (S1) strongly ridged, forming what appear to be paired central pits. S2 strongly convex, with basal groove coarsely ridged.

Color. Body black, with abundant whitish yellow markings as follows: Clypeus whitish except for transverse mesal spot black, apical margin brownish. Mandible brownish, basally white and teeth dark brown. All antennal articles black except scape whitish ventrally. White spot between antennae somewhat expended from middle. Ocular sinus with white spot. Temple with white spot. Pronotum with two triangular whitish spots. Metanotum medially with white spot. Tegula whitish except medial stripe. Parategula completely white. All femora brownish except apically white on fore and mid femur. All tibiae dorsally whitish with basal small brownish area in mid and hind tibiae and ventrally brownish. All tarsi brownish except pro and mid tarsi whitish with apically brownish spots. T1, T2 and S2 with white apical bands.

**Key to species of the genus *Subancistrocerus* from the Indian subcontinent**

(Modified from the key by Kumar, 2013)

1. Apical margin of metasomal T2 with reflexed and with regular series of large punctures……..... *reflexus* Giordani Soika

- Apical margin of metasomal T2 without reflexed and lack of large punctures……..2

2. Forewing hyaline with dark infuscation in the marginal cell; head, mesosoma and metasoma black with predominantly brownish yellow to yellowish brown maculations ..... *camicrus* (Cameron)

- Forewing hyaline without dark infuscation in the marginal cell; head, mesosoma and metasoma black with predominantly pale yellowish white or yellow maculations ..... 3

3. Clypeus black with sometimes a curved pale yellowish white line at base and rarely two pale yellowish white spots towards apex; tegula usually black, rarely with two yellow spots at anterior and posterior apices; metanotum black without yellow mark; legs predominantly black with yellowish white maculations..... ..... *sichelii* (de Saussure)

- Clypeus yellow except a brownish to blackish patch medially and a brown border on apical margin; tegula medially brown to black with two yellow spots; metanotum black with a yellow mark anteromedially; legs predominantly brown with yellow maculations..... 4

4. Pale markings bright yellow; second transverse carina of T1 weakly pronounced.... .....*venkataramani* Kumar

- Pale markings pale whitish; second transverse carina of TI strongly pronounced.... .....*pakistanensis* Qasim, Carpenter et Rafique **sp. nov.**

## ACKNOWLEDGEMENTS

We are grateful to the anonymous reviewer for their useful comments and suggestions that helped to improve this article. We are thankful to the Higher Education Commission (HEC) of Pakistan for funding under Start-Up Research Grant Program (SRGP), which made this study possible.

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*J. Entomol. Res. Soc., 24(3): 297-301, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2193* *Online ISSN:2651-3579*

# The Capture Effect of Yellow Sticky Traps of Different Tones on *Agalmatium bilobum* (Fieber, 1877) (Hemiptera: Auchenorrhyncha: Issidae) in Almond Orchards

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## ABSTRACT

This study was conducted to determine the effects of yellow traps of different tones against *Agalmatium bilobum* (Fieber, 1877) (Hemiptera: Auchenorrhycha: Issidae: Hysteropterinae) in almond orchards in Yurtbaşı District of Elazığ Province between May to July of 2018. In this study, planthopper individuals were caught in eight different yellow-toned traps and counted for 14 days. It has been determined that most planthopper individuals were attracted by the yellow color tone with trap codes 1016 (4) and 0279 (5). It is considered that this color tone is mostly effective for the attraction of *A. bilobum* in almond orchards. Accordingly, positive results can be obtained from using this kind of yellow tone trap by the producers to prevent the harm of *A. bilobum* to almonds. To the best of our knowledge, this is the first report related to the capture effect of yellow sticky traps on the Issidae species .

*Keywords:* Hysteropterinae, planthopper, sticky trap, colour tone, almond, Turkey.

Özgen, İ. & Gnezdilov, V.M. (2022). The capture effect of yellow sticky traps of different tones on *Agalmatium bilobum* (Fieber, 1877) (Hemiptera: Auchenorrhyncha: Issidae) in almond orchards. *Journal of the Entomological Research Society*, 24(3), 297-301.

*Received: December 04, 2021* *Accepted: August 26, 2022*

## INTRODUCTION

Almond production is considered to be of great economic importance in Turkey, however, there are many insect species harmful in almond orchards (Bolu, Özgen, & Çınar, 2005). In particular, *Agalmatium bilobum* (Fieber, 1877), a member of the planthopper family Issidae (subfamily Hysteropterinae) was recorded as harmful species in almond and fruit orchards (Bolu et al, 2005; Yayla, Kelten, Davarcı, & Salman, 1995; Tezcan & Zeybekoğlu, 2001; Kovancı, Gençer, Kovancı, & Akgül,2004), although Lodos (1986) did not treat its harm as economically important in Turkey. However, in California (USA) where *A. bilobum* was unintentionally introduced in the middle of the last century (Gnezdilov & O'Brien, 2006), this species harms by their mass egg-laying with abundant mud on grapevine stakes and adjacent telephone poles, trunks and branches of olive trees etc. (Caldwell & DeLong, 1948; Schlinger, 1958; Doering, 1958). In Eastern Europe, *A. bilobum* is recorded as polyphagous species (Logvinenko, 1975; Chumak, 2005). In Georgia, the species of the genus *Agalmatium* Emeljanov, 1971 were recorded as pests of grapevine and fruit trees (Batiashvili & Dekanoidzhe, 1967). In Turkey, another species of this genus, *Agalmatium flavescens* (Olivier, 1791), is harmful to olive trees in Mardin Province (Kaplan, 2019).

Thus *A. bilobum* potentially may be treated as harmful to cultivated plants and accordingly it is important to find methods of population control of this pest without using chemicals. In this study, the catching effect of different yellow color traps against *A. bilobum* was examined.

## MATERIAL AND METHODS

The study was carried out in three acres of almond orchards, which were damaged by *Agalmatium bilobum* in Elazığ (Yurtbaşı) Province, Turkey. The emergence of the egg packages of the species on almond trees was monitored and the traps were hung on the trees after March when these packages started to appear. The traps remained on the trees until the end of August. Adult stages of the species caught in the traps were counted. The period when the traps were hung on the trees coincided with the beginning of leafing in almonds and the end of summer. Traps were counted between early July and mid-July, when adult pests are most abundant in nature. This period coincides with the almond fruit harvest. The traps containing eight yellow-colored tones used in the study were hung on the 2 m branches of the trees (Fig. 1). RAL codes of the color tones used in the study are given in Table 1. Planthopper individuals were counted on the sticky traps. Individuals on the trap were taken from the trap after counting. The traps were checked twice a day and the total number was recorded daily. In order to determine the male/female ratio of individuals caught in different color codes, individuals carefully taken from yellow adhesive plates with the help of a fine-tipped needle were brought to the laboratory and examined.

## RESULTS

It has been determined that the most number of *Agalmatium bilobum* individuals was matched by the yellow color tone with a trap code of 1016 (4) and 0279 (5) (Fig. 2) Apart from these codes, the trap numbered 1021 showed the effect of attracting the pest. The percentage of male/female ratio of individuals caught in different color codes was determined as 40%/60%.

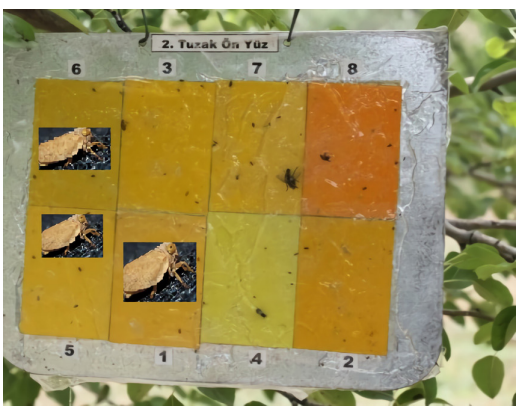


Fig. 1. Yellow stick traps with different ral codes on trees.

Table 1. Trap codes (Özgen et al, 2013; 2020).

| Number | Code | Trap Name | | Number | Code | Trap Name |
|---|---|---|---|---|---|---|
| **One** | 1021 | *Kadmiumgelb* | | **Five** | 0279 | *Scaniagelb* |
| **Two** | 1003 | *Signalgelb* | | **Six** | 1012 | *Zitronengelb* |
| **Three** | 1018 | *Zinkgelb* | | **Seven** | 1023 | *Werkehesgelb* |
| **Four** | 1016 | *Schwefelgelb* | | **Eight** | 1028 | *Melonengelb* |

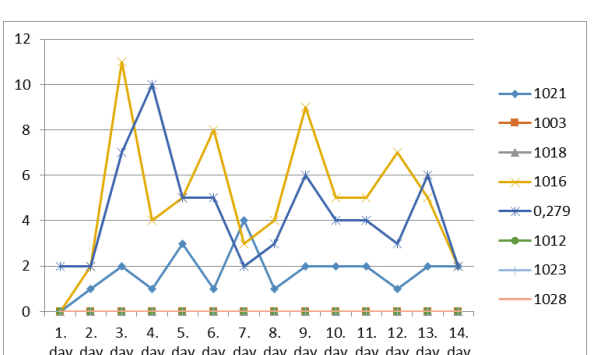


Fig. 2. Number of *Agalmatium bilobum* individuals caught with different tones of yellow traps.

## CONCLUSIONS

Previous studies have focused on the effect of these coded tones to attract some pests. Thus, the psyllids (Hemiptera), *Agonoscena pistaciae* Burckhardt et Lauterer, 1989 (Aphalaridae) was catched with the 1016 codes trap while *Cacopyslla pyri* (L., 1761) (Psyllidae) was catched with for the 1023 coded trap (Özgen, Ayaz, Mutlu, & Bolu, 2013; Özgen et al, 2020; Kavak, Özgen, & Güral, 2020). Moreover, *A. pistaciae* studies were moved to the next stage and the most effective code was determined by making changes to the effective code (Altun, Yetkin, Işık, & Özgen, 2018; Özgen et al, 2020).

In addition, the fact that the ratio of males and females of *Agalmatium bilobum* caught on the sticky plates was higher than the ratio of females to males indicates that it will be important in terms of comprehensive mass control efforts against the pest. As a result, these traps are important in terms of biotechnical control of these pests in olive and almond fields. The creation of mass capture optimization and efficiency testing will allow for obtaining better results in the future.

## ACKNOWLEDGEMENTS

The study of İnanç ÖZGEN was performed in the framework of The Scientific and Technological (TÜBİTAK) Research Project No: 118O124. We thank TÜBİTAK for the study grant. The study of Viladimir M. GNEZDILOV was performed in the framework of the Russian State Research project № 122031100272-3.

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*J. Entomol. Res. Soc., 24(3): 303-320, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2222* *Online ISSN:2651-3579*

# Host-Feeding Patterns of Mosquito Species in Aras Valley, Turkey

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## ABSTRACT

Identifying hosts of blood-feeding mosquito species is important to elucidate the role of vector species in disease transmission cycles. Herein, the aim was to identify the vertebrate hosts of mosquito species using the polymerase chain reaction-based reverse line blotting method. The mosquito species were *Anopheles maculipennis* sensu lato Meigen, 1818; *Aedes caspius* Pallas, 1771; *Aedes vexans* Meigen, 1830; *Culex theileri* Theobald, 1903; *Anopheles hyrcanus* Pallas 1771; *Culex pipiens* sensu lato Linnaeus, 1758; and *Culiseta annulata* Schrank, 1776, which were collected from the Aras Valley between July-August 2012 and June-September 2013. The analysis of mosquito blood-meal samples revealed several mammalian and avian host species. And also varying degrees of opportunistic mosquito feeding behavior in both birds and mammals were noted. These mosquito species fed on eight mammal species, i.e., humans, cows, sheep, horses, dogs, cats, goats, and porcupines, as well as avian species. Studies on the host-feeding patterns of blood-feeding vector arthropods may provide information about the vector potential of these species. However, the host-feeding patterns of the vector species are only one part of the vector capacity. To detect the exact potential of vector species, further studies are needed on vector competence for different pathogens.

*Key words:* Aras Valley, host-feeding patterns, mosquito species, reverse line blotting, vector biology.

Bedir, H., Demirci, B., & Vatansever, Z. (2022). Host-feeding patterns of mosquito species in Aras Valley, Turkey. *Journal of the Entomological Research Society*, 24(3), 303-320.
*Received: January 04, 2022* *Accepted: October 19, 2022*

## INTRODUCTION

The host-feeding patterns define the hosts that mosquitoes feed on in their natural habitats and can be influenced by numerous intrinsic and extrinsic factors such as host availability, host defences and mosquito host preference (Lyimo & Ferguson, 2019). The host-feeding patterns of mosquitoes are important for humans in the epidemiology of mosquito-borne pathogens. Female mosquitoes require blood to develop eggs; therefore, they obtain blood meal from a variety of hosts, such as humans and/or animals. The degree of contact with different vertebrate hosts may result in the adaptation and transmission of mosquito-borne parasites or pathogens to susceptible vertebrate species (Kilpatrick et al, 2013). Species exhibiting strong and genetic-based host selection behavior are the most important vectors of infectious diseases, and it has been predicted that this behavioral trait may have evolved parallel to host–parasite coevolution (Takken & Verhulst, 2013). The host-feeding patterns of mosquitoes are directed by various genetic and ecological factors, e.g., environmental conditions and host characteristics that affect host-seeking and selection patterns (Clements, 1992; Takken &Verhulst, 2013).

Determining the variety of vertebrate hosts as blood-meal sources for mosquitoes is essential to understand the dynamics of mosquito-borne diseases and to describe the role of various mosquito species in being a nuisance for humans and animals (Mukabana, Takken, & Knols, 2002).

In Turkey, various native mosquito species have been identified and demonstrated as potential pathogen and exotic vectors, including *Aedes albopictus* (Skuse, 1895) and *Aedes aegypti* (Linnaeus, 1762) (Akıner, Demirci, Babuadze, Robert, & Schaffner 2016). Virus screening of *Ae*. *albopictus* samples collected from several locations in the Black Sea Region of Turkey from 2016-2017 revealed the presence of West Nile virus (WNV), *Aedes flavivirus*, and the cell fusing agent virus. Merida-like virus Turkey, a recently described novel rhabdovirus, was also co-detected in a *Culex pipiens* sensu lato Linnaeus, 1758 pool that was positive for WNV (Akıner et al, 2019).

WNV is an extremely important virus that maintains its cycle between mosquitoes, birds, and mammals, such as humans and horses, as dead-end hosts because they do not develop sufficient quantities of the virus in their blood to infect mosquitoes that may feed on them, and horse-to-horse, horse-to-person, and person-to-person virus transmission does not occur.

Although several wild and domestic avian species are susceptible to WNV infection, birds often serve as natural reservoir hosts for WNV infections (Mihailović et al, 2020). In Europe, *Cx*. *pipiens* s.l. and *Aedes caspius* are the most important vectors associated with WNV, whereas *Culex theileri* Theobald, 1903 and *Anopheles maculipennis* sensu lato Meigen, 1818 are suggested to play a potential role in virus circulation (Calistri et al, 2010; Engler et al, 2013). WNV is common in Turkey, and *Cx*. *pipiens* s.l. and *Ae*. *caspius* Pallas, 1771 are potential vector species for WNV, similar to that in Europe (Ergünay et al, 2017; Öncü et al, 2018; Akıner et al, 2019). Furthermore, WNV-neutralizing antibodies were detected at a rate of 9.9% in ducks in Kars Province, which is located

at the border of our study region (Ergünay et al, 2014). The Aras Valley is located on one of the most important migratory bird routes in the world and provides nesting and breeding grounds for several bird species that migrate to the Middle East and Africa annually from Russia and the Caucasus (Şekercioğlu, 2008). The movement of infected migratory birds may play a role in the introduction and spread of arboviruses, such as WNV, on the European mainland (Calistri et al, 2010).

Previously, the presence of *Dirofilaria immitis* (Leidy, 1856) and *Dirofilaria repens* Railliet & Henry, 1911 (Nematoda: Filarioidea) DNA was shown in *An. maculipennis* s.l., *Ae. caspius*, *Ae. vexans*, *Cx. theileri*, *Cx. pipiens* s.l., and *Anopheles hyrcanus* Pallas 1771 in the Aras Valley (Demirci, Bedir, Taskin Tasci, & Vatansever, 2021). *D. immitis* and *D. repens* are present worldwide and cause cardiopulmonary and subcutaneous dirofilariasis in canines, felines, and other mammalian carnivores (Simon et al, 2012). Several mosquito species from different genera, such as *Anopheles*, *Aedes*, and *Culex*, have been identified as potential vectors for *Dirofilaria* spp. around the world (Simon et al, 2012).

The information presently available on the host-feeding patterns of mosquito species in Turkey is based on only two studies. One of them reported the host species preferences of blood-feeding mosquitoes belonging to *Cx. pipiens* s.l. collected from the Central Anatolia Kayseri province in Turkey (Korkmaz et al, 2017), and the other reported the host species preferences of blood-feeding mosquitoes belonging to *Cx. pipiens* s.l., Favre, 1903 and *Culex tritaeniorhynchus* Giles, 1901, from the Mediterranean and Aegean Regions in Turkey (Bursalı, 2020).

The present study was performed to determine the host-feeding patterns of mosquito species in the Aras Valley. The blood-meal hosts of seven mosquito species have been reported through blood-meal identification using reverse line blotting (RLB). This technique has allowed the successful identification of blood hosts of the tick *Ixodes ricinus* (Acari: Ixodidae) (Humair et al, 2007). RLB facilitates the simultaneous amplification of different species using polymerase chain reaction (PCR) and then distinguishes them using species-specific oligonucleotides. Therefore, RLB involves a hybridization step following PCR amplification, and it shows higher sensitivity than PCR alone. The use of chemiluminescent reagents instead of radioactive substances in imaging does not lead to harmful effects in people who perform this technique, thereby making the technique more user-friendly. In addition, the oligonucleotide-containing blot can be reused at least 20 times, thus providing an economic advantage for the users (Georges et al, 2001).

## MATERIALS AND METHODS

### Study region

Samples were collected from six villages in the Aras Valley, where the Iğdır Plain is located: Mürşitali, Küllük, Zülfikarköy, Pirli, Yukarıçamurlu, and Yukarıçıyrıklı (Fig. 1 and Table 2). The municipality authorities were informed about mosquito trapping, and sampling was performed on private lands after permission was obtained from the owner.

The Aras Valley, located in Northeast Anatolia, Turkey, is an important ecological corridor considered the entrance route for desert fauna into Anatolia (Demirsoy, 2002). The Aras Valley has high population densities of mosquito species due to the intense irrigation of agricultural lands and suitable climate conditions. Furthermore, sustainable agriculture and livestock activities host many cattle, sheep, goats, and poultry. Moreover, from a biological viewpoint, this region is an important basin located on the migratory route of several birds migrating from Russia and the Caucasus to the Middle East and Africa every autumn and returning in the spring (Şekercioğlu et al, 2011).


Fig. 1. Map showing the location of the six villages in the Aras Valley.

## Mosquito collection, identification, and storage

Mosquitoes were collected in 2012 (July-August) and 2013 (June-September) using New Jersey light traps (JW Hock Company, Gainesville, FL) and mouth aspirators. During the sampling period approximately 3 traps (i.e., close to the barns or in the barns, close to the houses and open areas neighboring croplands where people work) were operated for each village for one night per month. In addition, indoor resting mosquitoes were sampled with mouth aspirators by two experienced collectors from the walls and ceilings in the same sampling sites where light traps catches were conducted. Morphological identification of the Culicidae was done mainly using the electronic identification key of Schaffner et al (2001). The samples were stored at -80°C until DNA extraction.

## DNA extraction

Mosquitoes were scanned under a stereo microscope for abdomens that contained blood. The abdomens of blood fed female mosquitoes were excised and removed from the head/body sections using sterile forceps and placed in sterile 1.5-ml microtubes. Blood samples of vertebrates obtained from the Faculty of Veterinary Medicine, Kafkas University, with the approval of the Ethics Board, Kafkas University, Kars, Turkey, were

used as positive control samples. Genomic DNA was isolated from whole blood using the Purelink® Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

## Species-specific probes

Species-specific 5′-amino-linked oligonucleotide probes were designed by Abbasi et al, (2009) according to mammalian and avian vertebrate cytochrome b (CytB) sequences obtained from the direct sequencing of PCR products (Table 1).

Table 1. Species-specific oligonucleotide probes designed to identify different vertebrate blood types based on their hybridization with the CytB PCR product.

| Species | Oligonucleotide sequence |
|---|---|
| Human (*Homo sapiens*) | 5'-amino-ATG CAC TAC TCA CCA GAC GC |
| Cow (*Bos taurus*) | 5'-amino-ATT ATG GGT CTT ACA CTT T |
| Sheep (*Ovis aries*) | 5'-amino-TCC TAT TTG CGA CAA TAG CTTCCT |
| Goat (*Capra hircus*) | 5'-amino-ATA CAT ATC GGA CGA GGT CTA |
| Dog species | 5'-amino-CAG ATT CTA ACA GGT TTA |
| Horse/Donkey *(Equus asinus)* | 5'-amino-CTA CTT TTC ACA GTT TAG CTA CA |
| Domestic cat (*Felis domesticus*) | 5'-amino-CAT TGG AAT CAT ACT ATT |
| Porcupine (*Hystrix indica*) | 5'-amino-ACA CTG CCT ACA CAA CTA CA |
| Brown rat (*Rattus rattus*) | 5'-amino-CAG TCA CCC ACA TCT GC |
| Rock hyrax (*Procavia capensis*) | 5'-amino-CCT ATT CTT CGT ATG TCT TTA |
| General avian probe 1 (Avian) | 5'-amino-TAC ACA GCA GAC AC |
| General avian probe 2 (Avian) | 5'-amino-GCC TCA TTC TTC AT |
| Domestic chicken (*Gallus gallus*) | 5'-amino-CAT CCG GAA TCT CCA C |
| Rock pigeon (*Columbia livia*) | 5'-amino-ACT ACT CGC CGC ACA TTA |
| House sparraow (*Passer domesticus*) | 5'-amino-GAG ACG TCC AAT TCG GAT |
| Rock partridge (*Alectoris graeca*) | 5'-amino-TCA CCG GCC TCC TCC TA |
| Turkey (*Meleagris gallopavo*) | 5'-amino-CTT CTG TGG CCA ACA CAT |

## Polymerase chain reaction and reverse line blotting

Universal primers designed for the PCR amplification of 344 base pairs of the conserved region of CytB were used for amplification: Cyto1: 5′-CCA TCA AAC ATC TCA GCA TGA AA-3′ and Cyto2: 5′-biotin-CCC CTC AGA ATG ATA TTT GTC CTC-3′. The 50-µL PCR reaction included the following components: 5 U/µl Taq DNA polymerase (0.4 µl), 10 mM dNTPs (1 µl), 25 mM $MgCl_2$ (1.5 µl), each primer (1 µl), genomic DNA (2 µl), and $dH_2O$ (38.1 µl). The thermal cycling conditions were as follows: initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, primer extension at 72°C for 1 min; and final extension at 72°C for 8 min. The obtained PCR products were used as probes in RLB hybridization reactions.

The Biodyne C membrane (Pall Biosupport, Germany) was activated using 10 ml of 16% 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDAC; Carl Roth GmbH, Karlsruhe, Germany) for 9 min at room temperature. The activated membrane was washed with distilled water for 30 s and placed in the Miniblotter. The liquid remaining on the membrane (EDAC and distilled water) was aspirated. Each probe channel (150 µl), diluted to 50-1200 pmol/150 ml in 500 mM NaHCO3 (pH 8.4), was added to the Miniblotter channels, excluding the first and last; 4% ink diluted with 2X SSPE and 0.5% SDS was added into the first and last channels. After the loading process, the membrane was incubated for 5 min at room temperature and then aspirated. The membrane was washed with 100 ml 2X SSPE and 0.1% SDS at 60°C for 5 min. The oligonucleotide-immobilized membrane was rotated 90° in the opposite direction of the probes and placed in the Miniblotter, and the liquid was aspirated. Thereafter, 20 µl of PCR product was diluted with 150 µl 2X SSPE and 0.1% SDS. The diluted PCR products were denatured at 100°C for 10 min in a PCR thermal cycler. The denatured PCR products were immediately cooled on ice for 5 min to prevent reattachment of the DNA strands. Then, the PCR products were added to the Miniblotter channels and allowed to hybridize for 30 min at 75°C and for 60 min at 53°C in an incubator. The membrane was washed twice with 100 ml 2X SSPE and 0.5% SDS at 62°C in 10-min intervals, followed by incubation with 15 ml 2X SSPE and 0.5% SDS with 3 µl peroxidase-labeled streptavidin dilution at 42°C for 1 h in the hybridizer. Thereafter, the membrane was washed twice with 100 ml 2X SSPE and 0.5% SDS at 62°C in 10-min intervals and then washed twice with 100 ml 2X SSPE at 5-min intervals. Subsequently, the membrane was placed in 10 ml ECL detection liquid (Roche Diagnostics, Mannheim, Germany) for 5 min at room temperature. Finally, an X-ray image was obtained by placing the membrane between two acetate papers in a film cassette placed under Hyperfilm for 30 min. The formation of black spots upon the union of oligonucleotide probes to PCR products after the development of the film was considered positive. To control the sensitivity and specificity of the RLB, control RLB assays were performed with CytB products amplified from positive controls. Of the mammalian species-specific CytB products, only goat CytB PCR products showed faded cross-hybridization with sheep probes. While all other avian products hybridized with their own probes, only the partridge-specific probe showed some cross-reactivity with chicken DNA. However, while the chicken samples amplified with both Avian 1 and Avian 2 probes, the partridge samples amplified with only Avian 2 probes. Additionally, to avoid the consequences of human DNA contamination during collection, identification, extraction, etc., human signals were considered positive when they were strong.

To remove the PCR products from the membrane, it was washed twice in 100 ml 1% SDS at 80°C for 30 min. Then, it was rinsed in 240 ml 20 mM EDTA at room temperature for 15 min. Finally, the membrane was sealed in a plastic bag with 10 ml 20 mM EDTA and refrigerated at 4°C for future re-use.

## RESULTS

In total, 26,654 mosquito species were sampled during this study and of those, 11,403 were mosquitoes were visually determined to contain blood (Completely engorged, semi engorged and nearly digested blood fed females). Only completely engorged and semi engorged females were chosen for this study (916 mosquitoes). Of those, 655 (71.50%) representing seven species belonging to four genera were positive for CytB PCR (Table 2). The mosquitoes that were negative for PCR may have had dried blood in which DNA could not be extracted or < 0.1 pg DNA was present in the ingested blood (Abbasi et al, 2008). A gel image of CytB PCR-targeting DNA, extracted from blood-fed mosquitoes, is shown in Fig. 2. The results of mammalian and avian-derived blood meals are shown in Tables 4 and 5, respectively. The mosquitoes were found to feed on eight mammalian (i.e., humans, cows, sheep, horses, dogs, cats, goats, and porcupines) and several avian species. Opportunistic feeding behavior on both mammalian and avian hosts was detected at varying degrees, except for *Cs*. *annulata* and *An*. *hyrcanus* (Table 3). These two species fed only on mammalian species, whereas the other five species (*An*. *maculipennis* s.l., *Ae. vexans*, *Ae*. *caspius*, *Cx*. *pipiens* s.l., and *Cx*. *theileri*) fed on both mammalian and avian hosts.

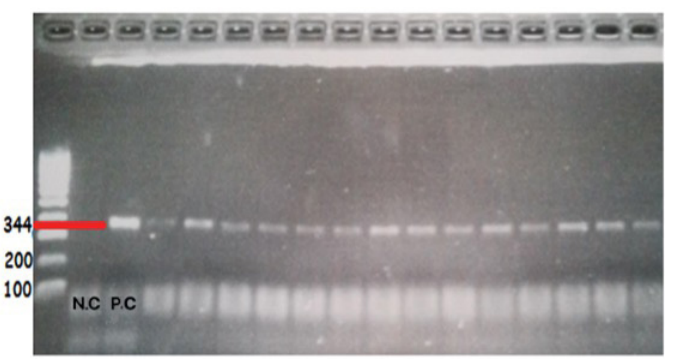


Fig. 2. PCR products of vertebrate cytochrome b sequences (344 bp) in the presence of mosquito species genomic CytB DNA- P.C-Positive control, N.C-Negative control.

The blood-meal samples belonging to mammalian and avian hosts predominantly included blood meal from humans (83.81%), followed by cow (53.28%), sheep (5.19%), partridge (4.88%), pigeon (4.12%), cat (1.06%) unidentified avian (0.61%), sparrow (0.45%), horse (0.30%), goat (0.15%), dog (0.15%), and porcupine (0.15%; Tables 4 and 5). Single and mixed blood-meal sources are shown in Table 6.

The pattern for single hosts was as follows: 219 (33.43%) of human blood, 97 (14.80%) of cow blood, 5 (0.76%) of sheep blood, 1 (0.15%) of horse blood, and 1 (0.15%) of pigeon blood. Mixed blood meal from two, three, and four hosts were also identified as follows: 234 (35.72%) of humans/cows, 24 (3.66%) of humans/sheep and humans/partridges, 6 (0.91%) of humans/pigeons, 4 (0.61%) of humans/avian, 1 (0.15%) of humans/goats, pigeons/partridges, and chickens/pigeons, 1 (0.15) of humans/cows/pigeons, 4 (0.61%) of humans/cows/sheep and humans/cats/pigeons, 2 (0.30%)

of humans/cows/cats, humans/cows/chickens, and humans/sheep/pigeons, 1 (0.15) of humans/cows/horses, humans/cows/dogs, humans/partridges/pigeons, humans/chickens/pigeons, humans/cows/partridges, and humans/cows/avian, 4 (0.61%) of humans/sheep/pigeons/partridges, and 1 (0.15%) of humans/cows/partridges/pigeons, humans/cows/cats/pigeons, and humans/pigeons/sparrows/porcupines (Table 6).

The seasonal proportion of human and cow-derived blood meal is presented in Fig. 3. The proportion of human-derived blood was significantly different between July and August, whereas the proportion of cow-derived blood was significantly different among all months except June and August ($p < 0.05$). The proportion of cow-derived blood decreased in July and August compared with that in June and September.

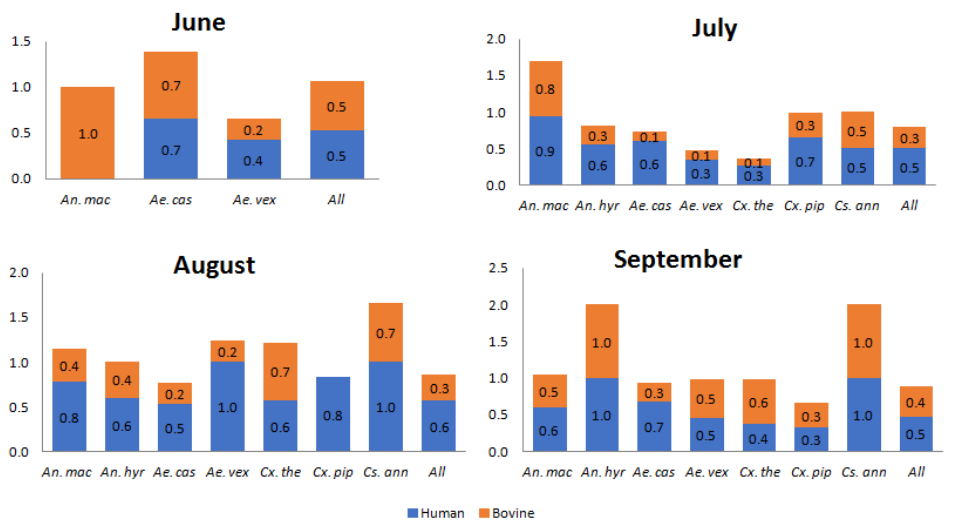


Fig. 3. The proportion of human and cattle-derived blood meals by months. *An. mac: An. maculipennis s.l., An. hyr: An. hyrcanus, Ae. cas: Ae. caspius, Ae. vex: Ae. vexans, Cx. the: Cx. theileri, Cx. pip: Cx. pipiens s.l., Cs. ann: Cs. annulata*, All: All species.

Table 2. CytB positive blooded mosquito species collected from six villages in the Aras Valley.

| Month/Year | Sampling villages | Coordinates | *An. maculipennis* s.l. | *An. hyrcanus* | *Ae. caspius* | *Ae. vexans* | *Cx. theileri* | *Cx. pipiens* s.l. | *Cs. annulata* |
|---|---|---|---|---|---|---|---|---|---|
| July/2012 | Yukarı Çıyrıklı | 40.12773° 43.60654° | 2 | 2 | | | | | |
| | Pirli | 40.06559° 43.78573° | 71 | | | 1 | | | |
| | Küllük | 40.02663° 44.13784° | | | | 1 | | | |
| August/2012 | Pirli | | 23 | 3 | | | | | |
| | Küllük | | 10 | | | | | | |
| June/2013 | Pirli | | 1 | | 4 | 9 | | | |
| | Zülfikarköy | 39.99232° 44.14654° | | | 25 | 38 | | | |
| | Yukarı Çıyrıklı | | | 1 | | | | | |
| July/2013 | Yukarı Çıyrıklı | | | 3 | 3 | | | | 1 |
| | Pirli | | | 1 | | | | | |
| | Mürşitali | 42.636222° 44.306610° | 20 | 2 | 26 | 21 | 21 | 5 | |
| | Yukarı Çamurlu | 39.94974° 44.39266° | 3 | 2 | 35 | | 28 | 1 | 2 |
| August/2013 | Küllük | | 7 | | | | | | |
| | Zülfikarköy | | | 2 | 8 | 19 | 2 | 1 | 2 |
| | Y. Çamurlu | | 2 | 1 | 30 | 11 | 32 | 3 | |
| | Yukarı Çıyrıklı | | 38 | | 2 | | | | |
| | Pirli | | | | | 10 | 11 | | |
| September/2013 | Küllük | | 3 | | | | | | |
| | Zülfikarköy | | | | | 11 | 8 | 3 | 2 |
| | Mürşitali | | 27 | 1 | 22 | 9 | 4 | | |
| | Yukarı Çamurlu | | | | 19 | | | | |
| Total | | | 207 | 18 | 174 | 130 | 106 | 13 | 7 |

Table 3. Number of the blood-meal sources by host class identified (mammals/avians) from 7 species of mosquitoes collected in the Aras Valley.

| Mosquito species | Total number of mosquitoes | Blood detected from only mammals | Blood detected from only avians | Blood detected from both mammals and avians |
|---|---|---|---|---|
| *An. maculipennis* s.l. | 207 | 174 | 0 | 33 |
| *An. hyrcanus* | 18 | 18 | 0 | 0 |
| *Ae. caspius* | 174 | 149 | 1 | 24 |
| *Ae. vexans* | 130 | 129 | 0 | 1 |
| *Cx. theileri* | 106 | 103 | 1 | 2 |
| *Cx. pipiens* s.l. | 13 | 10 | 1 | 2 |
| *Cs. annulata* | 7 | 7 | 0 | 0 |
| Total | 655 | 590 | 3 | 62 |

Table 4. Types of mammalian-derived (included all single and mixed feedings) blood-meal sources identified from mosquitoes collected in the Aras Valley.

| Mosquito species | Blood detected from humans | Blood detected from cows | Blood detected from sheeps | Blood detected from horses | Blood detected from goats | Blood detected from dogs | Blood detected from cats | Blood detected from porcupines |
|---|---|---|---|---|---|---|---|---|
| *An. maculipennis* s.l. | 185 | 135 | 26 | 0 | 1 | 0 | 5 | 0 |
| *An. hyrcanus* | 14 | 4 | 0 | 2 | 0 | 0 | 0 | 0 |
| *Ae. caspius* | 146 | 72 | 5 | 0 | 0 | 0 | 0 | 1 |
| *Ae. vexans* | 115 | 57 | 1 | 0 | 0 | 1 | 2 | 0 |
| *Cx. theileri* | 72 | 73 | 2 | 0 | 0 | 0 | 0 | 0 |
| *Cx. pipiens* s.l. | 11 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cs. annulata* | 6 | 5 | 0 | 1 | 0 | 0 | 0 | 0 |
| Total | 549 | 349 | 34 | 3 | 1 | 1 | 7 | 1 |

Table 5. Number of avian-derived (included all single and mixed feedings) blood meals identified from mosquitoes collected in the Aras Valley.

| Mosquito species | Blood detected from pigeons | Blood detected from sparrows | Blood detected from chickens | Blood detected from partridges | Other avian species |
|---|---|---|---|---|---|
| *An. maculipennis* s.l. | 25 | 0 | 2 | 12 | 0 |
| *Ae. caspius* | 1 | 2 | 0 | 18 | 4 |
| *Ae. vexans* | 0 | 0 | 0 | 1 | 0 |
| *Cx. theileri* | 1 | 0 | 2 | 1 | 0 |
| *Cx. pipiens* s.l. | 3 | 0 | 0 | 0 | 0 |
| Total | 27 | 2 | 4 | 32 | 4 |

Table 6. Single and mixed blood meal sources identified from mosquitoes collected in Aras Valley.

| Blood-meal sources | Species | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***An. maculipennis* s.l. (%)** | ***An. hyrcanus* (%)** | ***Ae. caspius* (%)** | ***Ae. vexans* (%)** | ***Cx. theileri* (%)** | ***Cx. pipiens* s.l (%)** | ***Cs. annulata* (%)** | **Total (%)** |
| Humans | 28 (4.27) | 9 (1.37) | 72 (11.0) | 71 (10.8) | 30 (4.58) | 7 (1.07) | 2 (0.30) | 219 (33.4) |
| Cows | 19 (2.90) | 3 (0.46) | 27 (4.12) | 15 (2.29) | 31 (4.73) | 1 (0.15) | 1 (0.15) | 97 (14.8) |
| Sheeps | 2 (0.30) | 0 | 1 (0.15) | 0 | 2 (0.30) | 0 | 0 | 5 (0.76) |
| Horses | 0 | 1 (0.15) | 0 | 0 | 0 | 0 | 0 | 1 (0.15) |
| Pigeons | 0 | 0 | 0 | 0 | 0 | 1 (0.15) | 0 | 1 (0.15) |
| Humans/cows | 101 (15.4) | 4 (0.61) | 45 (6.87) | 39 (5.95) | 40 (6.10) | 2 (0.30) | 3 (0.46) | 234 (35.7) |
| Humans/sheeps | 19 (2.90) | 0 | 4 (0.61) | 1 (0.15) | 0 | 0 | 0 | 24 (3.66) |
| Humans/goats | 1 (0.15) | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.15) |
| Humans/partridges | 6 (0.91) | 0 | 17 (2.59) | 1 (0.35) | 0 | 0 | 0 | 24 (3.66) |
| Humans/pigeons | 4 (0.61) | 0 | | 0 | 0 | 2 (0.30) | 0 | 6 (0.91) |
| Humans/sparrows | 0 | 0 | 2 (0.30) | 0 | 0 | 0 | 0 | 2 (0.30) |
| Humans/avians | 0 | 0 | 4 (0.61) | 0 | 0 | 0 | 0 | 4 (0.61) |
| Chickens/pigeons | 0 | 0 | 0 | 0 | 1 (0.15) | 0 | 0 | 1 (0.15) |
| Pigeons/partridges | 0 | 0 | 1 (0.15) | 0 | 0 | 0 | 0 | 1 (0.15) |
| Humans/cows/ sheeps | 4 (0.61) | 0 | 0 | 0 | 0 | 0 | 0 | 4 (0.61) |
| Humans/cows/horses | 0 | 1 (0.15) | 0 | 0 | 0 | 0 | 1 (0.15) | 2 (0.30) |
| Humans/cows/dogs | 0 | 0 | 0 | 1 (0.15) | 0 | 0 | 0 | 1 (0.15) |
| Humans/cows/cats | 0 | 0 | 0 | 2 (0.30) | 0 | 0 | 0 | 2 (0.30) |
| Humans/cows/ pigeons | 7 (1.07) | 0 | 0 | 0 | 0 | 0 | 0 | 7(1.06) |
| Humans/cows/ chickens | 1 (0.15) | 0 | 0 | 0 | 1 (0.15) | 0 | 0 | 2 (0.30) |
| Humans/cows/ partridges | 1 (0.15) | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.15) |
| Humans/cows/avians | 0 | 0 | 0 | 0 | 1 (0.15) | 0 | 0 | 1 (0.15) |
| Humans/sheeps/ pigeons | 2 (0.30) | 0 | 0 | 0 | 0 | 0 | 0 | 2 (0.30) |
| Humans/cats/ pigeons | 4 (0.61) | 0 | 0 | 0 | 0 | 0 | 0 | 4 (0.61) |
| Humans/chickens/ pigeons | 1 (0.15) | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.15) |
| Humans/partridges/ pigeons | 1 (0.15) | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.15) |
| Humans/cows/cats/ pigeons | 1 (0.15) | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.15) |
| Humans/cows/ partridges/pigeons | 1 (0.15) | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.15) |
| Humans/sheeps/ pigeons/partridges | 4 (0.61) | 0 | 0 | 0 | 0 | 0 | 0 | 4 (0.61) |
| Humans/pigeons/ sparrows/porcupines | 0 | 0 | 1 (0.15) | 0 | 0 | 0 | 0 | 1 (0.15) |
| Total | 207 | 18 | 174 | 130 | 106 | 13 | 7 | 655 |

## CONCLUSIONS AND DISCUSSION

Feeding pattern studies based on blood meal analysis can be biased in several ways such as sampling site location (host absence/presence and indoor/outdoor) and trapping methods, leading to a predominant host that does not necessarily reflect innate mosquito preference for that host (Fikrig & Harrington, 2021). In the present study, seven mosquito species were collected from six sampling sites located in the Aras Valley in the Northeastern Anatolia Region of Turkey, which fed on eight mammalian (humans, cows, sheep, horses, dogs, cats, goats, and porcupines) and several avian species. Humans were found to be the most common vertebrate hosts. Host similarities were detected in the host-feeding patterns, i.e., all the mosquito species in the area shared mostly humans and cows host species, as previously highlighted in similar studies from Iran (Shahhosseini et al, 2018), Germany (Börstler et al, 2016), and Switzerland (Schönenberger et al, 2016). Human-derived blood meals accounted for > 83% of all feedings, and human and cattle hosts comprised nearly three-fourths of all analyzed blood samples, similar to a study in Iran (Shahhosseini et al, 2018). A relatively high abundance of selected hosts present in the available host communities or a strong overlap in the host preference may be probable explanations for the detected host similarities between the mosquito species (Shahhosseini et al, 2018).

Moreover, the proportion of cattle-derived blood-feeding decreased in July and August compared to that in June and September. Although host selection is a genetic-based characteristic in response to certain host characteristics, host availability, and diversity in the environment where mosquito species exist considerably affect host orientation (Clements, 1992). Furthermore, mosquitoes have higher feeding success on inactive hosts (Day & Edman, 1984). Our study region has microclimate features based on its location and is influenced by the Mediterranean climate. Agriculture and animal husbandry are the main sources of income in the region, and residents work in orchards and fields throughout the day, particularly in the summer. In addition, barns are located close to the houses, and cattle are the main livestock reared in the region. Furthermore, cattle herding is moved to plateau lands between July and August, especially considering that the cattle population notably decreases during these months. The results of the present study showed that the proportion of blood-feeding mosquitoes on cattle hosts significantly decreased in July and August. Many factors, such as trap placement, availability of vertebrate host species, collection methods, and weather, can influence the mosquito species composition in the collection area, as well as the represented blood-meal hosts (Thiemann & Reisen, 2012). During our sampling process, only New Jersey light traps were used, and they were placed close to the barns or in the barns, as well as in open areas neighboring croplands where people work. In addition, indoor samplings were performed in houses and animal barns. Shahhosseini et al, (2018) detected certain differences between the host-feeding patterns of the Biogents Sentinel traps (BG trap; Biogents, Regensburg, Germany) Heavy Duty Encephalitis Vector Survey traps (EVS trap; BioQuipProducts, Rancho Dominguez, CA, USA) and handheld aspirators. Thus, more detailed studies with different collection methods and trap placements may have different results in the area. The density and diversity of vertebrate species were not

surveyed at the collection sites; thus, the significance of the relative representation of a species or lack thereof in the blood-meal host identification cannot be suggested. Rather than suggesting seasonal variation in host utilization, the results may indicate that mosquito species tend toward accessible hosts, and the sampled species tend to be present near hosts that are easily accessible, densely populated, and more tolerant to bite pressure, with minimum energy and maximum efficiency.

All mosquito species in the present study fed on humans, and/or other mammals, birds, and domestic mammals. These results may indicate the potential transmission risk of zoonotic pathogens, such as filarial nematodes (Azari-Hamidian et al, 2009), WNV (Nikolay, 2015), Sindbis virus (Brugman et al, 2017), and Usutu virus (Calzolari et al, 2013), which are transmitted between different mammals and birds, and Rift Valley fever virus (McDaniel et al, 2014), which causes outbreaks in cattle and humans. Our previous study conducted in the same area (the Aras Valley) showed that *An*. *maculipennis* s.l., *Ae*. *caspius*, *Ae*. *vexans*, *Cx*. *theileri, Cx*. *pipiens s.l. are* potential vectors of *D*. *immitis* and *D*. *repens* with DNA in head-thorax pools; *An*. *hyrcanus* is also a likely vector, but Dirofilaria DNA was only found in abdomen pools for the study area (Demirci et al, 2021). And also as we mentioned before WNV is common in Turkey, and *Cx*. *pipiens* s.l. and *Ae.caspius* are potential vector species for WNV (Ergünay et al, 2017; Öncü et al, 2018; Akıner et al, 2019). In Europe, *Cx*. *pipiens* s.l. and *Ae*. *caspius* are the most important vectors associated with WNV too, whereas *Cx. theileri* and *An. maculipennis* s.l. are suggested to play a potential role in virus circulation (Calistri et al, 2010; Engler et al, 2013). Furthermore, WNV-neutralizing antibodies were detected at a rate of 9.9% in ducks in Kars Province, which is located at the border of our study region (Ergunay et al, 2014). The four most common mosquito species in this study (*An*. *maculipennis* s.l., *Ae. caspius*, *Ae*. *vexans* and *Cx*. *theileri*) and also *Cx*. *pipiens* all fed on humans, non humans mammals and birds. Therefore these species may be classified as potential vectors between mammals and/or birds for the area. Field data on the host-feeding patterns of blood-feeding vector arthropods may provide information regarding the vector potential of these species. However, the host-feeding patterns of the vector species are only a part of the vector capacity. To detect the actual potential of a species as a vector, further studies are needed on vector competence for different pathogens.

The most abundant species, *An*. *maculipennis* s.l., commonly described to feed on mammals and birds (Brugman et al, 2017), showed opportunistic feeding behavior on a wide range of host species in the present study, which is in line with the literature (Danabalan et al, 2014, Schönenberger et al, 2016, Heym et al, 2019). The mixed feeding behavior of *Anopheles* mosquitoes could serve as a strategy to increase reproduction (McDaniel et al, 2014). Members of the *An*. *maculipennis* complex in the study were not identified at the species level. The sibling species in this complex may significantly differ in feeding preferences and therefore in vector capacity (Takken and Verhulst, 2013). The *An*. *maculipennis* complex has eleven members and the most important malaria vectors in the Palearctic region. In particular, *An*. *(Anopheles)* atroparvus van Thiel, 1927, *An*. *labranchiae* Falleroni, 1926 and *An*. *sacharovi* Favre, *1903* are considered the most important malaria vectors (BruceChwatt & de Zulueta 1980). Of these members,

four species were recorded in Turkey: *An. maculipennis* s.s. Meigen, 1818 (Diptera, Culicidae), *An. messeae* Falleroni, 1926 (Diptera, Culicidae), *An. melanoon* Hackett, 1934 (Diptera, Culicidae), and *An. sacharovi* Favre, 1903 (Diptera, Culicidae) (Kasap and Kasap, 1983; Parrish, 1959; Postiglione et al, 1973; Ramsdale et al, 2001; Şimşek et al, 2011). Furthermore, *An. hyrcanus* is one of the secondary vectors of malaria (WHO, 2018). Studies on the host preference of *An. hyrcanus* are limited; a study conducted in Bangladesh determined that the species fed on only mammalian hosts (Bashar et al, 2012). The Aras Valley, in addition to the Marmara region (north-western region of Turkey), the Mediterranean region (southern coast of Turkey) and low-lying parts of Southeast Turkey, is a suitable place in Turkey for the transmission of *Plasmodium falciparum* infection, as the temperature is convenient (Özbilgin et al, 2011). Thus, the presence of potential malaria vectors and their human host preference behavior indicate that the study area may be at risk for malaria and the importance of future studies to detect the presence of *Plasmodium* in the study area.

The species *Cx. pipiens* s.l. comprises two morphologically identical biotypes, *pipiens* and *molestus*, that can form hybrids. The biotypes of *Cx. pipiens* s.l. species were not identified in the present study. Biotype *pipiens* preferred birds, whereas biotype *molestus* preferred mammals, including humans (Byrne and Nichols, 1999; Dohm and Turell, 2001; Sanburg & Larsen, 1973). Several studies (Brugman et al, 2017, 2018; Shahhosseini et al, 2018) have shown that *Cx. pipiens* s.l. has a strong feeding preference for avian blood, and various laboratory (Fritz et al, 2015) and field studies (Apperson et al, 2004; Börstler et al, 2016) have shown the high mammalian orientation of this species, which is consistent with our results. A study conducted in Turkey found that this species fed on both avian and mammalian blood, and the preference for human blood was higher than that for other mammalian species, which is similar to our findings (Korkmaz et al, 2017). Another study showed that *Cx. pipens* s.l. fed only on birds and horses and had a strong feeding preference for avian blood (Bursalı, 2020). As mentioned previously, many factors, such as trap placement, vertebrate host species' availability, collection methods, and weather, may cause different results for the same species.

Host-feeding patterns for opportunistic mosquitoes are typically determined by the most abundant or readily available host species. Additionally, it reflects the fact that the host-feeding patterns of a single species can greatly vary in different regions, thereby affecting the role of species as a bridge vector in different regions. The global host range of *Ae. vexans* is primarily mammals and rarely birds (Fall et al, 2012; Molaei et al, 2006). In the present study, *Ae. vexans* was found to feed on mammalian hosts, and only one individual fed on avian blood. In Senegal, this species was shown to feed on mammals, but mostly on blood derived from horses (Biteye et al, 2019). Conversely, *Ae. caspius* is a highly anthropophilic species and is known to aggressively bite humans throughout the day (Napp et al, 2018; Soliman et al, 2016).

A study conducted in Kenya (Tchouassi et al, 2016) found that *Cx. theileri* feeds solely on mammals, whereas studies conducted in Spain and Iran found that they feed on both mammalian and avian species, with a wide host range, similar to our results (Martínez-De La Puente et al, 2012; Shahhosseini et al, 2018). In the present study,

*Cs*. *annulata* and *An*. *hyrcanus*, unlike other species, fed only on mammalian blood. Although this result was similar to that reported by previous studies conducted in the United Kingdom (Service, 1971) and Portugal (Osório et al, 2012) for *Cs*. *annulata*, another study conducted in the United Kingdom, showed that this species also fed on avian blood (Brugman et al, 2017).

Although a wide range of studies have been conducted on mosquitoes in Turkey, the analysis of host-feeding patterns of mosquitoes is a highly unattended area of research. This is the most detailed study of the host-feeding patterns of mosquitoes in Turkey to date. The results indicate a strong overlap of host taxa with the highest frequencies for humans and cows. The overlap of host species' feeding patterns among mosquito species indicates that host selection is highly variable and may be correlated with the presence/abundance of the hosts. Vector competence is described the capacity of a mosquito to obtain the pathogen and support its transmission. Thus, these results suggest that most species in the area can potentially transmit pathogens between mammals and between birds and mammals. Therefore, for more concrete conclusions about the host-feeding patterns of mosquito species, it is necessary to give attention to the abundance and availability of host species in the field. A better understanding of the host-feeding patterns of mosquitoes will advance our comprehension of mosquito-borne disease epidemiology and enable the development of effective disease control strategies, such as by targeting host-specific vector species more accurately.

## ACKNOWLEDGEMENTS

This work was supported by a grant from the The Scientific and Technological Research Council of Turkey (TUBITAK) under grand no; KBAG 114Z011.

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*J. Entomol. Res. Soc., 24(3): 321-329, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2254* *Online ISSN:2651-3579*

# New Records of Gall Midges (Diptera: Cecidomyiidae) from Iran

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## ABSTRACT

Based on the materials which recently collected from west Azerbaijan province/Iran, two genus of gall midges including *Giraudiella* Rübsaamen, 1915 and *Stenodiplosis* Reuter, 1895 and seven species of them namely, *Baldratia salicorniae* Kieffer, 1897; *Cystiphora taraxaci* (Kieffer, 1888); *Dasineura plicatrix* (Loew, 1850); *D. teucrii* (Tavares, 1903); *G. inclusa* (Frauenfeld, 1862); *Lasioptera flexuosa* (Winnertz, 1853) and *S. bromicola* (Marikovskii & Agafonova, 1961) are reported for the first time for Iranian fauna. In addition, the species *Halodiplosis araratica* Mirumian, 1991 is a new record for west Azerbaijan province' fauna. Some photos of galls on their host plants and its distribution data are given.

*Key words:* New records, Cecidomyiidae, Iran.

Karimpour, Y., Skuhrava, M., Lotfalizadeh, H., & Razmi, M. (2022). New records of gall midges (Diptera: Cecidomyiidae) from Iran. *Journal of the Entomological Research Society*, 24(3), 321-329.
*Received: February 25, 2022* *Accepted: July 19, 2022*

## INTRODUCTION

Gall midges are small and fragile flies belonging to the order Diptera, the suborder Nematocera, and the family Cecidomyiidae. The family Cecidomyiidae is one of the largest families of the Diptera in the world (Skuhravá, Skuhravý, & Brewer, 1984). Many phytophagous species of gall midges are known for the typical galls they produce on host plants during the larval development. The shape of galls is used to identify different species of the family. Although commonly known as gall midges, but only two - thirds of the known species are phytophagous gall-maker. Other species are phytophagous without makings gall, mycophagous, predator and parasitoids (Gagné, 1981).

In west Azerbaijan (Azebaijan-e Gharbi) province, north western Iran, a total of 18 species of the family Cecidomyiidae were reported during last ten years (Karimpour & Skuhravá, 2012; Hashemi Khabir, Sadeghi, Harris, & Hanifeh, 2012; Skuhravá, Karimpour, Sadeghi, Gol, & Joghataie, 2014; Skuhrava & Karimpour, 2017; Karimpour & Skuhravá, 2022). From which four species are associated with various species of *Salix* spp. (Salicaceae) and the larvae of one species namely, *Dasineura rosae* (Bremi, 1847) live in the folded leaflets of *Rosa canina* L. (Rosaceae). The larvae of other species grow in flower, leaf buds and leaves of herbaceous plants. More recently a new genus and species of gall midges namely, *Cephalaromyia* Skuhravá, 2017 and *Cephalaromyia capituli* Skuhravá, 2017 were described from Urmia environs. The larvae of *C. capituli* develops in flower heads of *Cephalaria microcephala* Boiss. (Caprifoliaceae) and forming a small gall in it (Skuhrava & Karimpour, 2017). The aim of this survey is to search and identify more species of gall midges' fauna of west Azerbaijan province.

## MATERIAL AND METHOD

Galled host plant samples were collected and put in the polyethylene bag and transferred to the laboratory during the years 2019-2020 from six districts of west Azerbaijan province - Iran:

KHOY, Pïrkandï village: 38º 43′ 30″ N, 45º 06′ 36″ E, 1000 m asl.

KHOY, (in front of Mahlazan village, next to Khoy-Tabriz Road: 38º 39′ 03″ N, 45º 05′ 27″ E, 1019 m asl.

KHOY, (in front of Khoy airport, next to Khoy-Salmas road: 38º 24′ 17″ N, 44º 54′ 01″ E, 1192 m asl.

URMIA, Urmia University Campus: 37º39′14″ N, 44º58′35″ E, 1360 m asl.

URMIA, Kelïsä Kandï village (Sir Mountain): 37º29′08″ N, 45º01′31″ E, 1638 m asl.

URMIA, Shïrü Kandï village (Qasemlü valley): 37°18′02″ N, 45°07′10″E, 1433 m asl. (Fig. 1).

After transferring to laboratory, plant materials were kept separately in glass boxes (50 × 40 × 80 cm) covered with muslin, fixed with a rubber band, until the emergence of adults. Soft sand was poured on the bottom of the box to a height of 2 cm so that

the species that pupate in the soil have a place for pupation. Boxes were checked daily for collection of emerging gall midge adults for a 25-30 days. Obtained adults were put in vials with 75% ethanol and kept for identification. Emergence dates of adults were recorded. The specimens were identified using the keys written by Skuhravá (1997).

Specimens are deposited in the collection of the Department of Plant Protection, Faculty of Agriculture, Urmia University, Iran. Voucher specimens, both on slides and in ethanol, are deposited in the second author's collection at the Bítovská 1227/9, CZ-140 00 Praha 4, Czech Republic. The species are listed in alphabetical order by the genus.


Fig. 1. Sampled area and the distribution map of identified gall midge's species. 1-KHOY, Pïrkandï village, *Stenodiplosis bromicola,* 2-KHOY, Mahlazan village, *Lasioptera flexuosa*, *Baldratia salicorniae* and *Halodiplosis araratic*a, 3-KHOY, in front of Khoy airport, *Baldratia salicorniae* and *Halodiplosis araratica*, 4-URMIA, Urmia University Campus, *Cystiphora taraxac*i, 5-URMIA, Kelïsä Kandï village, *Dasineura teucrii* and *Giraudiella inclusa,* 6-URMIA, Shïrü Kandï village, *Dasineura plicatrix.*

## RESULTS AND DISCUSSION

Collecting galled plant materials and keeping them under appropriate laboratory conditions led to the gradual emergence of adult gall midges that were responsible

for causing galls in collected plant species. These gall midges belonged to seven genera and eight species as follow.

***Baldratia salicorniae* Kieffer, 1897**

**Distribution**: Along the coasts of Black Sea, Mediterranean Sea, several islands in these seas, southern France, Spain, Portugal, southern parts of England, Italy, former Yugoslavia, Morocco, Algeria, Tunisia, Libya, Egypt, Eritrea and Israel (Skuhravá & Skuhravý, 2021; Dorchin & Freidberg, 2008).

**Material examined:** 2♀, 1♂, host plant: *Salsola dendroides* Pall. (Chenopodiaceae) 3-9.09.2020. Galls on stems were collected in 29th August 2020 from Khoy (in front of Khoy airport, next to Khoy-Salmas road. We also obtained 2♀ and 2♂ during 15-21 April 2021 from overwintering galls. Overwintering galls were collected in 15th March 2021 from the same area.

**Host plants**: the species induce galls on leaves of *Salicornia fruticosa* (L.), *Salicornia europaea* L. and *Sarcocornia perennis* (Mill.) (Dorchin & Friedbberg, 2008; Gagné & Jaschhof, 2021).

**Remark**: So far, five species of this genus have been reported from Iran (Skuhravá et al, 2014). Of which, the host plant of *Baldratia aelleni* Möhn, 1969 belong to *Suaeda* Forssk. ex J.F. Gmel. genus namely, *S. microphylla* Pall.. *Baldratia aelleni* known only from Iran and described by (Möhn, 1969) based on materials that collected from Tehran province, Central Alborz, vicinity of Simin Dasht village by Manoutcheri & P. Aellen in 4 September 1948.

The *B. salicorniae* and *S. dendroides* association is new.

***Cystiphora taraxaci* (Kieffer, 1888)**

**Distribution**: widespread in Europe, Pakistan and introduced to Canada for target weed biological control (Gagné & Jaschhof, 2021).

**Material examined:** 7♀, 5♂; host plant: *Taraxacum officinale* L. (Asteraceae). 18-26. 09. 2021. Galls on lamina were collected in 14th September 2021 from Urmia, Urmia University campus.

**Host plants**: All species of *Cystiphora* cause galls on the leaves of tribe Cichorieae (Asteraceae) plants. The orange larvae of *C. taraxaci* cause several flat circular pustule galls on lamina with dark red or purple margin in old blisters on *T. officinale*. Larva are active under a translucent epidermis of blister.

**Remark:** This is second species of *Cystiphora* which recorded from Iran. *Cystiphora sonchi* (Vallot, 1827) has already been reported from Iran/west Azerbaijan province (Karimpour & Skuhravá, 2012).

***Dasineura plicatrix* (Loew, 1850)**

**Distribution**: widespread in Europe and North Africa, Türkiye (Gagné & Jaschhof, 2021). North America (Sinclair et al, 2009).

**Material examined**: 3♀, 2♂; host plant: *Rubus caesius* L. (Rosaceae) during the days from 20-26.07.2020 (Fig. 2a,b). The plant materials were collected from vicinity of Shïrü Kandï village, (Qasemlü valley) in 17th July 2020.

**Host plants**: *Dasineura plicatrix* is an oligophagous gall midge species which recorded from *R. caesius* and other species of *Rubus* L. (Barnes, 1926; Alford, 1984; Darvas, Skuhravá & Andersen, 2000). It has been reported as a minor pest of blackberry (*R. laciniatus* Willd.), loganberry (*R. loganobaccus* L.H. Bailey), and raspberry (*R. idaeus* L.) in northern Europe (Darvas, Skuhravá & Andersen, 2000).


Fig. 2. a) Folded and distorted leaves of European dewberry caused by larvae of *Dasineura plicatrix* and b) larvae of *D. plicatrix* inside the folded leaf of *R. caesius* (scale bar: 1mm, infested stem around 20 cm).

### *Dasineura teucrii* (Tavares, 1903)

**Distribution**: former Czechoslovakia, Belgium, Switzerland, England, France, Germany, Portugal, Italy, Greece, Romania, Spain and former Yugoslavia (Skuhravá & Skuhravý, 2021).

**Material examined:** 3♀, 3♂; host plant: *Teucrium polium* L. (Lamiaceae) during 11-17.07.2020 (Fig. 3a,b). The plant materials were collected from Sir Mountain, near the Sir church, vicinity of Kelïsä Kandï village in 8th July 2020.

**Host plants:** Gregarious orange larvae (Fig. 3b) cause leaf bud galls on *Teucrium scorodonia* L., *T. lusitanicum* Lam., *T. salviastrum* Schreber, *T. lusitanicum* Schreber, *T. scorodonia* L., and *T. chamaedrys* L. (Lamiaceae)

**Remark:** Eight species of the genus *Dasineura* Róndani, 1840 were reported from Iran (Skuhravá et al, 2014a). By introducing of these two species, their number reaches ten species.

The association between *D. teucrii* - *T. polium* is new.

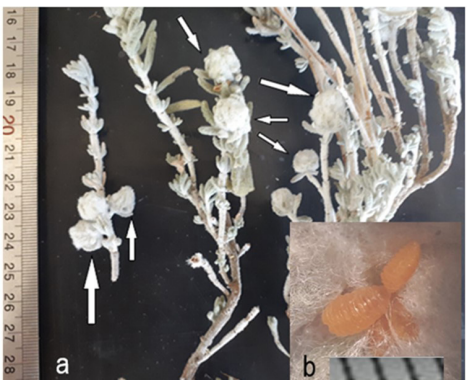


Fig. 3. a) leaf bud galls of *D. teucrii* on *Teucrium pollium* and b) larvae of *D. teucrii* (scale bar: 1mm)

### *Giraudiella inclusa* (Frauenfeld, 1862)

**Distribution**: widespread in the Palaearctic region and immigrant in north America, Eastern USA (Skuhravá & Skuhravý, 2021).

**Material examined**: 11♀, 9♂; host plant: grain-like galls in the stems of *Phragmites australis* (Cav.) (Poaceae) (Fig. 4a,b) during the days from 8-19.05.2020. The plant materials were collected from Sir Mountain, near the Sir church, vicinity of Kelïsä Kandï village in 26th March 2020.

**Host plant**: *Phragmites australis.*

**Remark:** This is the first record of genus *Giraudiella* from Iran.


Fig. 4. a) The common reed stem infested by *Giraudiella inclusa* and b) the same stem after dissection and grain-like galls in it.

### *Halodiplosis araratica* Mirumian, 1991

**Distribution:** Iran/Mazandaran Province, Amol (Delarostagh) (Skuhravà et al, 2014) and Armenia (Mirumian, 2011).

**Material examined**: 5♀, 3♂; host plant: *Salsola dendroides* Pall. (Chenopodiaceae) (Fig. 5a,b). Galls on stems were collected in 10th September 2021 from Khoy (in front of Khoy airport, next to Khoy-Salmas road and next to Khoy-Tabriz Road (16th km), vicinity of Mahlazan village in 18th September 2021.

**Remark**: Two species of gall midges namely *B. salicorniae* and *H. araratica* were associated with *S. dendroides* in west Azerbaijan province.

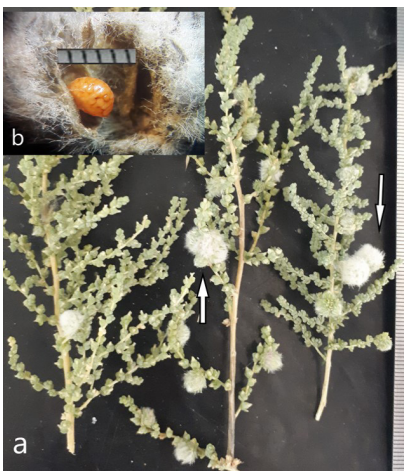


Fig. 5. a) furled galls of *H. araratica* on the leaves of *S. dendroides*, b) the larva of *H. araratica* inside the gall (scale bar: 1mm).

### *Lasioptera flexuosa* (Winnertz, 1853)

**Distribution**: former Czechoslovakia, Austria, Hungary and Czech Republic (Gagné & Jaschhof, 2021).

**Material examined**: 4♀, 5♂; host plant: stems of *Phragmites australis* (Cav.) (Poaceae) during the days from 23-29.09.2020. The plant materials were collected from Khoy, next to Khoy-Tabriz Road (16th km), vicinity of Mahlazan village in 18th September 2020.

**Remark**: With report of *G. inclusa* and *L. flexuosa*, in this paper the number of gall midge species that are associated with common reed in Iran (west Azerbaijan province) reaches three species. Because, the species *Asynapta phragmitis* (Giraud, 1863) has already been reported from Iran, west Azerbaijan province, Urmia (Skuhravá et al, 2014).

Till now, two species of *Lasioptera* namely, *L. carophila* Löw, 1874 and *L. umbelliferarum* Kieffer, 1909 were reported from Iran (Skuhravá et al, 2014). By introducing of *L. flexuosa,* their number reaches three species.

### *Stenodiplosis bromicola* (Marikovskii & Agafonova, 1961)

**Distribution**: Germany, Poland, Ukraine, Russia (Europe), W Asia; immigrant to Canada (Saskatchewan, Alberta), USA (Oklahoma, Missouri, Wisconsin, Maryland). (Gagné & Jaschhof, 2021).

**Material examined**: 1♀ was obtained from inflorescences of *Setaria glauca* (L.) (Poaceae) in 28.09.2020. The plant materials were collected from Khoy, vicinity of Pirkandi village in 18th September 2020, sugar beet field.

**Host plants**: *Bromus inermis* (Poaceae).

**Remark**: This is the first record of genus *Stenodiplosis* from Iran. The *S. bromicola-S. glauca* association is new.

## CONCLUSION

Till now, 38 genera and 67 species of gall midges were known from Iran (Skuhravá et al., 2014; Moeinadini, Madjdzadeh, & Skuhravá, 2017; Hadi, Lotfalizadeh, Kazemi, & Skuhravá, 2018). In this study, 2 genera and 7 species of gall midges are reported for the first time from Iran. By introducing these 2 genera and 7 species, the number of known gall midge fauna of Iran reaches 40 genera and 74 species. Of which the highest number - 26 species of gall midges - was recorded in the north-western part of Iran, in West Azerbaijan Province, 12 species in Khorasan Province and 11 species in the northern parts of Iran near the Caspian Sea (in Golestan Province) and the remaining 25 species have been reported from other provinces of Iran. Therefore, more than 38% of the known species of gall midges in Iran are from West Azerbaijan province. Also, the number of known genera of gall midges in West Azerbaijan province reaches 14 genera.

Among the species reported from Iran in this research, only two species namely, *C. taraxaci* and *G. inclusa* have been reported from Pakistan and Iraq (Gagné & Jaschhof, 2021), respectively, but there is no report on the distribution of the remaining species in neighboring countries of Iran.

The finding of gall midges, *B. salicorniae, D. plicatrix, D. teucrii* and *L. flexuosa* in west Azerbaijan province extends our knowledge on the geographical distribution of these species into the border of Eastern Palaearctic region in Western Asia. With these findings it may be presupposed that researchers will discover in future studies of gall midge fauna will be able to find the species in adjacent countries of Iran.

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*J. Entomol. Res. Soc., 24(3): 331-338, 2022* *Short Communications*
*Doi: 10.51963/jers.v24i3.2265* *Online ISSN:2651-3579*

# Reduced Toxicities of Insecticides Against Brown Planthopper (*Nilaparvata lugens* Stål) Collected from Rice Fields in Bangladesh

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## ABSTRACT

The brown planthopper, *Nilaparvata lugens* (Stål), caused severe yield losses in rice production in many Asian countries. Chemical control is the key component of the integrated pest management tool to control rice brown planthopper (BPH). However, reduced toxicity of insecticides against this pest has been reported. We investigated the BPH mortality rate and the evolution of insecticide resistance in BPH collected from Bangladesh to commonly used insecticides by the rice-stem dipping method. Both the BPH laboratory strain and field populations were used in this study. The mortality rate of the Gazipur field population ranges from 15% to 80%, after exposure to imidacloprid in different concentrations. The highest mortality was found in response to dinotefuran followed by chlorpyrifos and etofenprox. The mortality rate increased after the time of exposure in relation to the increased concentrations of the insecticides. Additionally, the selected BPH strain shows a higher level of resistance to imidacloprid (resistance ratio = 179.45-fold), while other field populations show low to moderate levels of resistance. Increased detoxification enzyme activity, cytochrome P450 monooxygenase, was found in imidacloprid-resistant BPH. This study provides important information regarding the reduced toxicities of frequently used insecticides to control BPH.

*Keywords:* Brown planthopper, chemical control, mortality, resistance ratio, mechanism.

Datta, J., Kabir, M.M.M., & Ali, M.P. (2022). Reduced toxicities of insecticides against brown planthopper (*Nilaparvata lugens* Stål) collected from rice fields in Bangladesh. *Journal of the Entomological Research Society*, 24(3), 331-338.

*Received: March 16, 2022* *Accepted: October 17, 2022*

## INTRODUCTION

Rice is a major cereal crop around the globe after wheat. However, the production of rice faces constraints by several factors such as floods, drought, weeds, pests, and diseases (Balasubramanian, Sie, Hijmans, & Otsuka, 2007; Haque et al., 2021). Diseases like bacterial blight, blast, and destructive pests like brown planthopper (BPH), stem borer, and green leafhopper hampering rice production in many Asian countries (Seck, Diagne, Mohanty, & Wopereis, 2012). Among them the BPH (*Nilaparvata lugens* Stål) is considered a key rice pest that caused significant yield losses in major rice-growing areas, such as China, Vietnam, Thailand, Japan, Korea, India, Bangladesh, and the Philippines (Hereward et al, 2020; Nguyen et al, 2021). The sap-sucking nature of the pest caused the complete drying of the rice plants, widely known as 'hopper-burn' (Datta et al, 2021a). Chemical control is the primary pest management strategy to manage this pest. The key chemical insecticides used to control BPH are neonicotinoids, pyrethroids, carbamates, organophosphates, and insect growth regulators (Fujii et al, 2020; Datta & Banik, 2021). However, the toxicity against BPH has been reduced and the pest also evolved low to high levels of resistance to commonly used insecticides (Datta et al, 2021b). According to Arthropod Pesticide Resistance Database BPH evolved resistance to 33 active ingredients of insecticides (APRD, 2021). Neonicotinoids are the principal group of insecticides for BPH control followed by pyrethroids, carbamates, and organophosphates (Matsuda, Ihara, & Sattelle, 2020). Islam et al (2009) mentioned the first 'hopper-burn' in rice fields of Bangladesh in 1976 and an outbreak of BPH was reported in 1983. Although, chemical insecticide is widely used to control rice pests including BPH in Bangladesh, very limited studies have been carried out to monitor the toxicities of insecticides commonly used in this region. Therefore, this study aimed to investigate the mortality rates of BPH in response to common insecticides, the toxicities of different insecticides, and the metabolic mechanism that evolved in BPH.

## MATERIALS AND METHODS

### Insect

A total of four BPH strains were used in this study. Two field populations were collected from the rice fields of Gazipur and Dinajpur, and two laboratory strains (one susceptible and one resistant selected strain) of BPH were reared for several generations. The susceptible strain (Sus) was reared in the laboratory without exposure to any insecticides, in contrast, the resistance selected strain was reared with exposure to imidacloprid for eight generations. All of the collected insects were reared on susceptible rice variety, BR3, in a controlled environment (16/8 hour light/dark photoperiod).

### Insecticides

Details of insecticides tested against brown planthopper were shown in Table 1. The commercial-grade insecticide was directly dissolved in water in a series of five to seven concentrations (mg/L). Water solution without any chemicals was considered as the control.

Table 1. Insecticides and their classifications used to monitor mortality rates.

| Insecticide group | Insecticides | Purity | Classification[1] | Source |
|---|---|---|---|---|
| Neonicotinoids | Imidacloprid | 20% SC | 4A | Auto Crop Care Ltd., Bangladesh |
| | Dinotefuran | 20% SC | | ACI Formalities Ltd., Bangladesh |
| Organophosphate | Chlorpyrifos | 22% SC | 1B | Auto Crop Care Ltd., Bangladesh |
| Pyrethroids | Etofenprox | 30% EC | 3A | New Agro industries Ltd., Bangladesh |

[1]Classification has been done by Insecticide resistance action committee (IRAC)

### Bioassay

The biological assay of the rice-stem dipping method was performed with the fourth-instar nymphs of BPH. Rice plants at the tillering stage were used in monitoring mortality rates and detecting levels of resistance. Rice plants were washed thoroughly and then kept to dry. These rice stems are then dipped into an insecticide solution for up to 30 seconds and let dry. For each replicate, twenty-five nymphs of BPH were transferred onto a plastic bottle containing the treated rice plant by a homemade aspiration device. There were at least three replications for each concentration. Mortality rates of one field population (Gazipur) were assessed after 48, 72, and 96 hours (hr) of insecticide treatments. The nymphs were considered as dead if they were unable to move after gentle pushing with a soft brush.

### Enzymatic assay

Biochemical assays were done to measure enzyme activities in the imidacloprid-resistant BPH strain. The activities of cytochrome P450 monooxygenase (P450) were determined according to the previously described method with the minor modification (Asperen, 1962). The protein concentration of all the enzyme solutions was determined by the Bradford method (Bradford, 1976).

### Statistical analysis

Abbott's formula was used to correct the percent mortality rate of treated BPH (Abbott, 1925). The program DPS software (Data Processing System, version v15.10) was used for probit analysis to determine $LC_{50}$ (median lethal concentration) value. The resistance ratio (RR) was calculated by dividing the $LC_{50}$ value of a field population by the corresponding $LC_{50}$ value of susceptible BPH strain. The resistance ratio was classified as: RR>100-fold as a high resistance level, 10<RR<100-fold as a moderate resistance level, 5<RR<10-fold as a low resistance level, and RR<5-fold as susceptibility.

## RESULTS

### Mortality rates of BPH to different insecticides

Mortality rates of the BPH field population (Gazipur) to imidacloprid, dinotefuran, chlorpyrifos, and etofenprox, in 48, 72, and 96 hr of insecticide application have been

presented in Fig. 1. The mortality rates of BPH when exposing to the insecticides increased with the time of treatment. The highest mortality rate was found in BPH exposed to dinotefuran after 96 hr of insecticide application followed by imidacloprid, chlorpyrifos, and etofenprox. In all four insecticides exposure, the mortality rate was significantly higher in the highest insecticide concentration and after 96 hr of insecticide application.

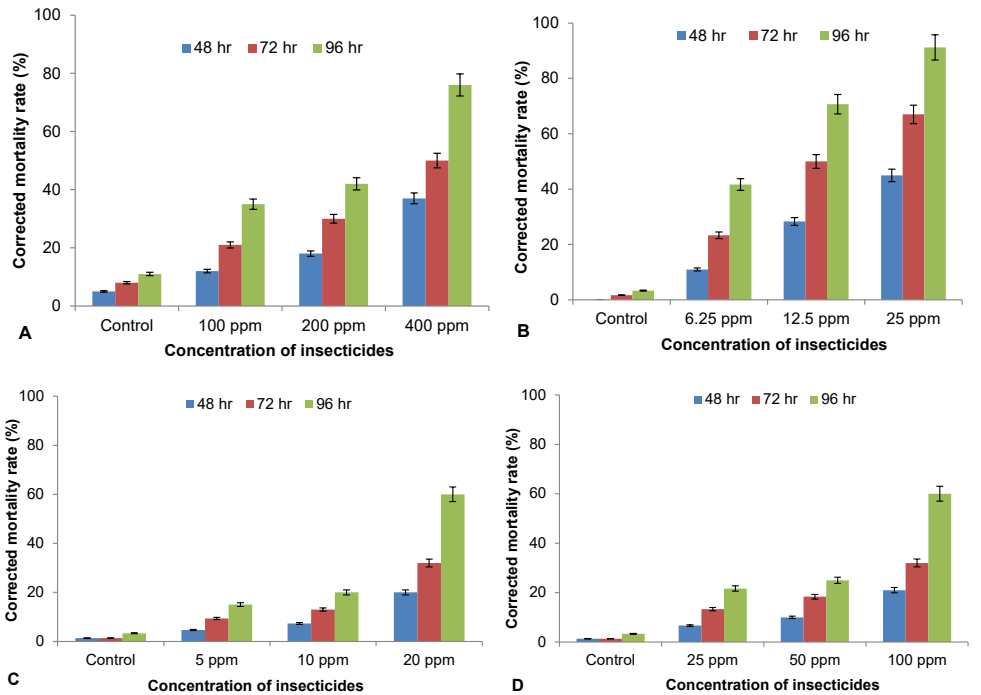


Fig 1. Mortality rates of Gazipur BPH field population after the application of insecticides, (A) imidacloprid, (B) dinotefuran, (C) chlorpyrifos, (D) etofenprox, at different doses.

## Relative toxicity of different insecticides against BPH

The $LC_{50}$ values of laboratory susceptible BPH strain to imidacloprid, dinotefuran, chlorpyrifos, and etofenprox were 1.54, 0.98, 1.25, and 9.37, respectively. The $LC_{50}$ values of laboratory susceptible BPH strain were used as a baseline to calculate the resistance ratio of BPH (Table 2). The BPH strain, selected with imidacloprid for eight generations, showed a higher level of resistance to imidacloprid (RR = 179.45-fold), followed by dinotefuran (RR = 22.93-fold) and etofenprox (RR = 15.06-fold). The Gazipur field population showed low to moderate levels of resistance to different insecticides, with the lowest resistance to chlorpyrifos (6.31-fold), and a moderate resistance to imidacloprid (63.42-fold). Additionally, the Dinajpur BPH field population showed low levels of RR to etofenprox (8.53-fold), chlorpyrifos (9.30-fold), and dinotefuran (9.70-fold), while a moderate level of resistance to imidacloprid (87.12-fold) (Table 2).

Table 2. Toxicity of different insecticides to lab strain and field collected BPH population.

| Strains | Insecticides | Slope±SE | $LC_{50}$ | RR |
|---|---|---|---|---|
| Lab[1] | Imidacloprid | 1.29±0.17 | 1.54 | 1 |
| | Dinotefuran | 2.21±0.20 | 0.98 | 1 |
| | Chlorpyrifos | 1.77±0.19 | 1.25 | 1 |
| | Etofenprox | 1.89±0.15 | 9.37 | 1 |
| Selection[2] | Imidacloprid | 2.42±0.30 | 276.36 | 179.45 |
| | Dinotefuran | 2.44±0.21 | 22.47 | 22.93 |
| | Etofenprox | 2.08±0.19 | 141.09 | 15.06 |
| Field - Gazipur | Imidacloprid | 2.12±0.25 | 97.67 | 63.42 |
| | Dinotefuran | 1.79±0.24 | 13.34 | 13.61 |
| | Chlorpyrifos | 1.02±0.20 | 7.89 | 6.31 |
| | Etofenprox | 1.78±0.30 | 82.67 | 8.82 |
| Field - Dinajpur | Imidacloprid | 2.39±0.32 | 134.17 | 87.12 |
| | Dinotefuran | 1.40±0.24 | 9.51 | 9.70 |
| | Chlorpyrifos | 1.52±0.30 | 11.62 | 9.3 |
| | Etofenprox | 1.43±0.20 | 79.89 | 8.53 |

[1]reared for more than 15 generations in controlled environment without any contact to insecticides

[2]BPH strain selected with imidacloprid for eight generations

## Enzymatic activity of resistant BPH

Metabolic enzyme activity of P450 in imidacloprid-resistant BPH strain has been measured and compared with laboratory susceptible strain (Fig. 2). More than double fold increase (2.14-fold increase) in P450 activity in BPH resistant strain has been found compared to susceptible strain.

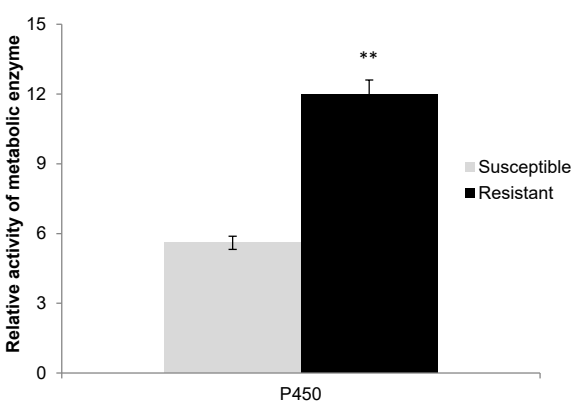


Fig. 2. Relative activity of P450 enzyme in imidacloprid-resistant BPH compared to laboratory susceptible BPH strain. Significant difference was indicated by asterisks above the error bar determined by student t-test ($P < 0.05$).

## DISCUSSION

Currently, chemical control is considered as the key measure to manage major crop pests including BPH, small brown planthopper, stem borer, rice bugs, etc (Sparks & Lorsbach, 2017; Datta et al, 2021a). However, continuous and heavy use of chemical insecticides caused reduced toxicities of commonly used insecticides against BPH. Neonicotinoids became the more efficient and popular insecticides to control brown planthopper across the rice-producing countries in Asia (Ihara & Matsuda, 2018). Particularly, in Bangladesh more farmers are using neonicotinoids, insect growth regulators, and combinations of different insecticides to control BPH despite their reduced toxicity (Datta et al, 2021a). However, an increasing trend of resistance to commonly used insecticides was recently reported, which is worrying for other rice-producing countries (Khan, Kaleem-Ullah, Siddiqui, & Ali, 2020; Chakrabarty et al, 2022). In this study, we determined the mortality rates of BPH after exposure to insecticides (48, 72, and 96 hr of exposure), and also toxicities of insecticides and the metabolic enzyme activities have been measured. Our previous study showed low to extremely high levels of resistance has been evolved in BPH collected from three locations in Bangladesh. Therefore, in this study, we have collected BPH field populations that are different from previous locations. The mortality rates of BPH increased with time after exposure to insecticides, which is also related to the concentration of insecticides (Fig. 1). Banks, Banks, Joyner, & Stark (2008) presented a model with time-varying mortality rates with the levels of insecticide exposure. The mortality rates increased with the time after exposure but the rates may decline or stable after a certain period of time. There might be different reasons for the dissimilar mortality rates of BPH. One reason might be due to the mode of action and the other is the susceptibility of the pest to insecticides.

Extremely high levels of resistance to imidacloprid, and moderate resistance to dinotefuran had evolved in BPH collected from Bangladesh in 2020 (Datta et al, 2021a). The development of insecticide resistance to neonicotinoids in BPH has also been reported across Asian countries (Ding et al, 2013; Sanada-Morimura et al, 2019). In our investigation, we also found that BPH developed low to moderate levels of resistance to chlorpyrifos, etofenprox, dinotefuran, and imidacloprid (Table 2). The BPH has the potential to develop a higher level of resistance when constantly exposed to certain insecticides, for instance, we found a high level of resistance in BPH to imidacloprid after selection for several generations. Resistance in BPH to chlorpyrifos increased after selection for a particular period of time and the resistance ratio was significantly higher in resistant BPH strain (Lu et al, 2017).

Many studies identified metabolic resistance mechanism, increased detoxification enzyme, as the main mechanism that contributes to resistance development in BPH. Increased enzyme activities of P450, GST, and EST were found responsible for resistance development in BPH (Datta & Banik 2021). We found increased activities of P450 in imidacloprid resistant-BPH. This indicates that enhancement of P450 activity may contribute to imidacloprid resistance development in BPH. This result agrees with the previously reported data where P450 was suggested as the

key detoxification enzyme (Datta et al, 2021a). However, we did not investigate the molecular mechanisms and the candidate genes that encoded enzymatic activities involved in resistance development in BPH. Further studies are required to understand the molecular mechanism involved in resistant BPH strains.

Our data indicated that mortality rates increased as the exposure time and concentration of insecticides increased. There are also possibilities of rapid resistance development in BPH. To assess the mechanisms, we measured the metabolic enzyme activities and found enhanced P450 activity in imidacloprid-resistant BPH. This study outlines the importance of regular monitoring that is required to understand the trend of reduced toxicities. The finding will assist in future studies to understand the molecular mechanism of resistance development.

## DECLARATIONS

Funding This research was funded by USAID-sponsored Partnerships for Enhanced Engagement in Research (PEER) Program's Cycle 6 project, entitled "Ecosystem services in a changing climate; assessing critical services in Bangladesh rice production landscapes". PEER is implemented by U.S. National Academy of Sciences (NAS) via USAID and NAS Prime Agreement No. AID-OAA-A-11 -00012.

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval The authors did not perform any study with animals or humans' participants to prepare this manuscript.

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*J. Entomol. Res. Soc., 24(3): 339-345,2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2272* *Online ISSN:2651-3579*

# *Chrysosoma nellyae* (Diptera: Dolichopodidae), a New Fly Species Discovered from Bohol Island, Philippines

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## ABSTRACT

A new fly species, *Chrysosoma nellyae*, has been recently discovered in Bohol, Philippines. Aside from the metallic blue head with black antennae which are reminiscent of *Chrysosoma* species, its thorax is metallic blue with a greenish and brown prescutellar patch, scutellum brown, blue green border with hair like bristle. In addition, based on the similarity of the male genitalia, cercus morphology, and legs, the new species appears to be related to *C. crinicorne.* To increase the accuracy of species identification, DNA barcoding of the mitochondrial cytochrome oxidase I gene was employed and further inferred for its phylogenetic relationship with other *Chrysosoma* species.

*Key words*: Diptera, *Chrysosoma*, phylogenetic analysis, DNA barcoding, Bohol Island.

Jose, R.P., Deocaris, C.C., Andres, M.I., & Alinsug, M.V., (2022). *Chrysosoma nellyae* (Diptera: Dolichopodidae), a new fly species discovered from Bohol Island, Philippines. J*ournal of the Entomological Research Society*, 24(3), 339-345.

Received: March 30, 2022 Accepted: November 23, 2022

## INTRODUCTION

Dolichopodidae, or the long-legged flies are known to be the largest dipteran family with about 7,500 species being described globally (Bickel, 2009). *Chrysosoma* (Dolichopodidae) is a large and complex genus of the Old-World tropics where major species groups are defined (Bickel & Lian-Meng, 1996). There are 84 dolichopodid species recorded from the Philippines, of which only 36 species have their type locality in the country (Ramos, Meier, Nuneza, & Grootaert, 2018). According to Delfinado and Hardy (1969), at least ten species of *Chrysosoma* were reported and described by Frey (1925) in the country, and three of these were found in Leyte, Samar, and Dumaguete. Ramos et al. (2018) listed 12 species of *Chrysosoma* in the country. Considering the underestimation of the true diversity in the Philippines based on a global inventory of Dolichopodidae (Yang, Zhu, Wang, & Zhang, 2006), we explored the species diversity in Bohol Island, a mega-biodiversity hotspot in the Philippines, which has never been dealt with comprehensively. Using morphological and DNA barcoding approaches, we report a new fly species collected in the forests of Rajah Sikatuna Protected Landscape (RSPL) of Bohol Island.

## MATERIALS AND METHODS

### Taxonomy

With permission to sample and later an issuance of a gratuitous permit from Department of Environment and Natural Resources-Region VII (Wildlife GP 2019-11, series of 2019), collections were made at the Magsaysay Park, Bilar, Bohol with malaise traps deployed in areas with minimum disturbance: Trap 1 (9.70431°N, 13 24.1239 E) and Trap 2 (9.70359°N, 124.1252°E) from June to August 2016. The traps were allowed to remain open during the entire sampling period and the bottles containing 70% ethanol alcohol were changed every week. Collected specimens were sorted into various families and placed in separate vials with screw caps and labels/codes for molecular work. Sorting of specimens was based on the guidebook by Oosterbroek (1998) and verified by taxon-specific experts from the National University of Singapore (NUS).

Images of *C. nellyae* were acquired using a Dun Inc. Passport II microphotography system, fitted with a Canon 65mm 5X MPE lens. The images were compiled using Zerene Stacker and digitally processed using Photoshop CS5.

To confirm new species identification, taxonomic experts at NUS compared the undetermined specimens with specimens from a digital reference collection, a physical reference collection of specimens used for taxa identification that needs to be identified routinely by taxonomists from different backgrounds and updated identification tools for dipterans (Ang et al., 2012).

## Molecular data

### DNA extraction and sequencing

DNA extraction from the entire leg of a sample specimen was done using QuickExtract™ DNA Extraction solution (Lucigen) and based on the methods of Wong et al (2014). Primer pairs (IDTdna) used to target the mitochondrial COI gene for PCR amplification were as follows: Forward: GGWACWGGWTGAACWGTWTAYCCYCC and Reverse: TAWACYTCWGGRTGNCCRAARAAYCA. PCR conditions were set at the following: initial denaturation at 95°C (3 mins); 1 cycle of 94°C (1 min), annealing 47°C (1 min), and 72°C (1 min), followed by 40 cycles with final extension set at 72°C (5 mins). The PCR products were purified using Sure Clean (Bioline), quantified in equimolar ratios using Nanodrop (Quiagen), and pooled prior to library preparation and Next-Generation Sequencing with Illumina MiSeq and HiSeq 2500 sequencing platforms. Sequencing libraries were prepared by AITbiotechusing the TruSeq Nano DNA Library Preparation Kit (Illumina), according to the manufacturer's protocol. Illumina MiSeq runs were provided by AITbiotech with the use of MiSeq Reagent Kit v3 (2 X 300 bp read lengths) while HiSeq runs were provided by SCELSE with HiSeq 2500 System and Rapid SBS Kit v2 (2 X 250 bp read lengths). The respective partial sequence of the COI mitochondrial DNA has been submitted to NCBI GenBank for data referencing with Accession No. ON023659.

### Phylogenetic analysis

The DNA analysis pipeline was based on the method of Meier, Wong, Srivathsan, & Foo (2016). Paired-end reads were merged using PEAR 0.9.6 (Zhang, Kobert, Flouri, & Stamatakis, 2014). Reads from each PCR product were assigned to their corresponding specimen using a uniquely labeled primer pair, and the dominant read was identified as the specimen barcode. A Python script was implemented (Srivathsan & Meier, 2012) to demultiplex the data, count the number of reads per sample, identify and group identical reads of these amplicons into sets, identify the dominant set of reads and combine it with otherwise identical length-variants, and counting the number of reads in the largest identity set, and comparing it with the count of the set with the second-highest number of reads. For purposes of quality control, barcoding of a particular sample was successful if: (i) the total read count was > 50x, (ii) the total barcode count was > 10x, and (iii) and the most dominant read was at least five times that of the second most dominant read (Meier et al., 2016).

The sequences were then used for NCBI-BLAST (Basic Local Alignment Search Tool) to search for sequences that match (>97% identity) the taxa. The top twenty specimens with the highest percentage match (86-88%) were retrieved for phylogenetic analyses. All retrieved sequences were aligned using MAFFT version 7 (https://mafft.cbrc.jp/alignment/software/). Phylogenetic inferencing of the aligned sequences was done using MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Evolutionary relationships of each taxon were inferred using the Neighbor-Joining method (Saitou and Nei 1987) with 1000 bootstrap support. Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei, & Kumar, 2004).

## RESULTS AND DISCUSSION

### *Chrysosoma nellyae* sp. nov.

**Type material.** Philippines, island of Bohol, province of Bilar, Magsaysay Park, 295 meters asl, 9.70431°N, 13 24.1239 E, 08.30.2006, 1♂ (Fig. 1), leg. RP Jose, Entomology Laboratory, National University of Singapore (after the photo was taken, the specimen was stored dry, withered, and stored in a collection).

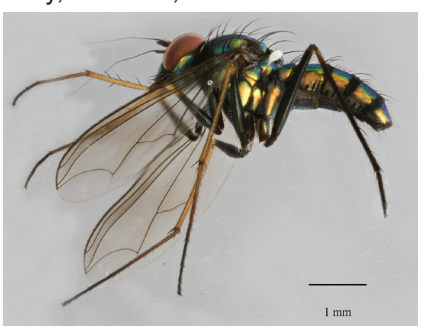


**Fig. 1.** Images of a new fly species, *Chrysosoma nellyae,* discovered from Bohol Island**.** Holotype, male (P20F99R24) showing the (A) dorsal and (B) lateral habitus of the specimen. Scale bar was set at 1 mm.

**Dimensions.** Body Length: 4.0-5.0 mm, Height: 5.0-6.0 mm, Wings: 10.0-11.0 mm.

**Description.** Male. Atenna: Arista with basal segment black, base of apical aristal segment pale becoming black towards tip. Palpus light brown elongate with bristling. Head: brightly metallic blue with yellowish metallic near antenna; Thorax: is metallic blue. with a greenish and brown prescutellar patch. Scutellum brown, blue green border with hair like bristle. Wings: Its spotless hyaline wing, length of 4.5 mm, has dark brown cross veins; Abdomen: With first tergite to last tergite is metallic blue in color with blue green and yellow brown marginal patch at the center. Legs: Black femur with shades of metallic blue; light brown tibia; dark brown tarsus; and long slender legs, relative length of foreleg (femur: 3.3 mm; tibia: 3.5 mm; tarsus: 3.1 mm); mid leg (femur: 4.0 mm; tibia: 5.5 mm; tarsus: 3.5 mm) hind leg (femur: 4.5 mm; tibia: 6.0 mm; tarsus: 2.5 mm). An image of the index species is shown in Fig. 1.

**Diagnosis.** Ratio of length to height of 1$^{st}$ flagellomere to length of arista, 0.8: 0.8: 13.4. Ratio of epistome to clypeus height, 28: 22. Four or five dorso-central setae. Fore coxa yellow; middle and hind coxae dark. Middle tibia and tarsus without erect pubescence and long setae. Length ratio of fore tibia to tarsus (segments from first to third), 125: 89: 19: 15. Length ratio of middle tibia to tarsus (segments from first to fourth), 115:39:26:12: 10. Length ratio of hind tibia to tarsus (segments from first to fifth), 225: 104: 39: 26: 15: 11. Wing without distinct spots. Ratio of parts of costa between R2+3 and R4+5 to those between R4+5 and M1, 21: 6. Ratio of crossvein m-cu to apical part of CuA 1, 53: 30. Ratio of apical part of M1 +2 (fork-handle) to M2, 61: 37. Cross vein m-cu sinuate; M1 gently curved; M2 poorly developed. Anal lobe well developed; anal angle acute. Length: body 5.1 mm, wing 5.3 mm. Morphologically,

the unidentified species is related to *Chrysosoma crinocorne*. Nonetheless, this new species differs morphologically with thorax is metallic blue with a greenish and brown prescutellar patch. Scutellum brown, blue green border with hair like bristle.

**Molecular characterization**

To further confirm the identification of the unknown Diptera, DNA barcoding was conducted using mitochondrial CO1 as the gene marker. A partial sequence of 342bp from the CO1 region was amplified and compared to other dipteran DNA sequences. Two DNA-sequence based approaches were undertaken for species discrimination and identification namely, the "best match" approach where the DNA sequence is directly compared to all species barcodes and the tree-based identification (Meier, Shiyang, Vaidya, & Ng, 2006; Blaxter et al, 2005; De Salle, Egan, & Siddall, 2005; Barrett & Herbert 2005).

For the "best match" approach, the DNA sequence of the unknown species was queried for exact match or highly similar sequences in the NCBI GenBank database using the BLAST search engine. Based on the results, there were no exact matches. The closest and most similar known sequence is *Chrysosoma crinocorne* sharing only 87% similarity to the unidentified species. The 13% variation in its DNA sequence is significant enough to indicate that *Chrysosoma nellyae* is new compared to the collection of DNA barcodes in the database.

To further discriminate and identify *Chrysosoma nellyae* using its DNA barcode, tree-based identification approach using the Neighbor-Joining tree was applied. An unidentified species or query sequence is considered successfully identified when it clusters to conspecific sequences. Clustering sequences by similarity utilizes pair wise distances. Thus, we retrieved and analyzed 20 other mitochondrial COI sequences of different fly species sharing the highest similarity with our unidentified species.

Fig. 2 displays a phylogenetic tree with 20 of its closest species that share 86-87% similarities to illustrate the evolutionary relationships within the taxa. Our result of the Neighbor-Joining method with 1000 bootstrap support further confirms that the unidentified species is closely related to *Chrysosoma crinicorne* with bootstrap support of 46% and shares high relatedness to *Chrysosoma vittatum* clustering as one group. Both *C. crinicorne* and *C. vittatum* have been documented to be present in the Philippines together with the other 10 recorded *Chrysosoma* species (*C. annuliferum*, *C. chrysoleucum*, *C. excitatum*, *C. fistulatum*, *C. fusiforme*, *C. pelagica*, *C. philippinense*, *C. proliciens*, *C. schistellum*, and *C. terminatum*) (Yang et al, 2006). Unfortunately, only *C. crinicorne* and *C. vittalum* have partial COI DNA sequences deposited in NCBI for molecular identification both emerging to share a high similarity as well as relatedness to *Chrysosoma nellyae*. Although it is expected that *Chrysosoma bearni* should have clustered together with the rest of the *Chrysosoma* species, *C. bearni* was never recorded to be found in the Philippines; thus, it may have variable sequences or morphological phenotypes that are not reminiscent of Philippine *Chrysosoma* species. Numbers next to branches of the Neighbor-Joining tree indicate the percentage of replicate trees where associated taxa clustered together in the bootstrap test.

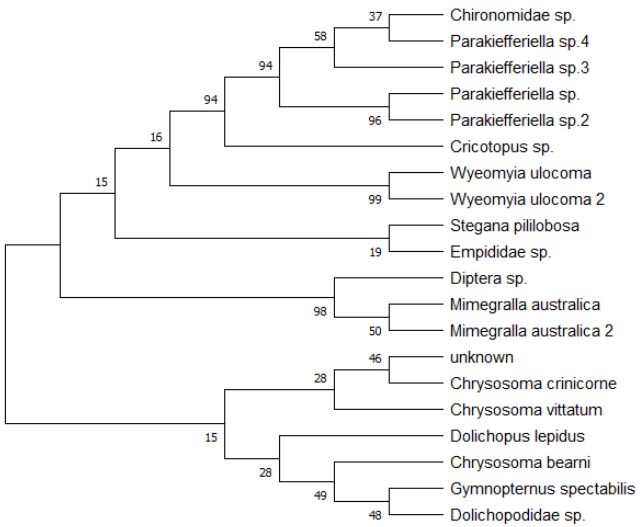


**Fig. 2.** Phylogenetic analysis using MEGA X. The top twenty species sharing the highest similarity to the DNA sequence of *Chrysosoma nellyae* sp. nov. were inferred for phylogenetic analysis using the Neighbor-Joining method with 1000 bootstrap support. *Chrysosoma nellyae* sp. nov. is more closely related to *Chrysosoma crinicorne* with a bootstrap support of 46% and shares high relatedness to *Chrysosoma vittatum* clustering as one group. Numbers next to branches indicate percentage of replicate trees where associated taxa clustered together in the bootstrap test.

Thus, by combining the morphological descriptors with molecular characterization, the unidentified fly species is undoubtedly classified under the genus *Chrysosoma* and is a new species ubiquitously abundant in Bohol Island, a mega-biodiversity hotspot in the Philippines. The insect will be the 13th species of *Chrysosoma* discovered in the Philippines.

Etymology. The species name is in memory of the mother of RPJ.

## ACKNOWLEDGMENT

We are grateful to the technical support and guidance of Dr. Yuchen Ang and Dr. Rudolf Meier from National University of Singapore and Dr. Maosheng Foo from Lee Kong Chian Natural History Museum; Ms. Gladys R. Cabelin for sharing her expertise and technical assistance; the Department of Environment and Natural Resources-Region VII for the gratuitous permit in specimen collection; Bohol Island State University and Dr. Olga Nunez of Mindanao State University-Iligan Institute of Technology. RPJ was a fellow of the Philippine's Commission on Higher Education K to 12 Graduate School Sandwich Program.

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*J. Entomol. Res. Soc., 24(3): 347-352, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2278* *Online ISSN:2651-3579*

# Descriptions of Two New Species of *Camptoptera* Foerster, (1856) from India (Hymenoptera: Mymaridae)

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## ABSTRACT

Two new species of *Camptoptera* Foerster (Hymenoptera: Mymaridae) are described based on the materials collected from Indian states: *C. ayezae* Anwar & Zeya sp. nov. and *C. sadhui* Anwar & Zeya sp. nov. The type specimens are deposited in the Insect Collections, Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

*Key words*: Chalcidoidea, fairyfly, egg parasitoids, taxonomy, new species India.

Anwar P. T., Zeya, S. B., Usman, S.U., Khan, F. R., Ahmad, Z., & Ghramh, H. A. (2022). Descriptions of two new species of *Camptoptera* Foerster, (1856) from India (Hymenoptera: Mymaridae). *Journal of the Entomological Research Society,* 24(3), 347-352.

Received: April 18, 2021 Accepted: October 10, 2022

## INTRODUCTION

*Camptoptera* Foerster (Hymenoptera: Chalcidoidea) is one of the most extensively studied genera of Mymaridae in Oriental region. The genus can easily be identified by their petiolated body, long antenna and narrow wings. However, species level identification will always be a daunting task because its taxonomy is based largely on sculpture of the mesosoma which requires an expert hand and high level concentration in slide preparation.

Most elaborative and revisionary works of the genus were provided by Triapitsyn (2014) for Palaearctic fauna and Anwar et al. (2020) for Oriental fauna. Viggiani (1978) and Subba Rao (1989) described several species from India and Sri Lanka which were later reviewed by Triapitsyn (2017) and Anwar et al. (2020). More recently, Anwar et al. (2021a) added three species of the genus from India (described one and recorded two known Taiwanese species). Huber et al. (2021) synonymised the genus *Eofoersteria* Mathot (1966) under *Camptoptera* and treated it as a subgenus of *Camptoptera* and Anwar et al. (2021b) provided the first ever description of the males of *Camptoptera* (*Eofoersteria*). The genus presently contains 33 species from Oriental region and 30 species from India (including two species described here as new) which makes it most diverse regions of the world in terms of *Camptoptera* species.

## MATERIAL AND METHODS

The study is based on the materials collected from different states of India. The specimens were collected using various trapping techniques and were preserved in 80% ethanol. After noting their body colour and lengths slides were prepared for their further identification following the Anwar et al. (2020). The terms mentioned in the text follow Zeya & Hayat (1995) and Gibson (1997). The measurements other than body lengths are relative and taken from slide mounts. All the measurements are in micrometers (µm). Photographs were taken from slide mounted specimens with a Leica® DFC295 digital camera attached to a Leica® DM 2500 compound microscope. Photographic plates were prepared using Adobe Photoshop® 7.0. All the type materials were deposited at the Insect Collections Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

The following abbreviations are used in the text: F1-6 = funicular segments 1-6; mps = multiporous plate sensillum or sensilla; MT = malaise trap; SN = sweep net; YPT = yellow pan trap.

The following acronym is used for the specimen depository:

ZDAMU = Insect Collections, Department of Zoology, Aligarh Muslim University, Aligarh, India.

## RESULTS

### Taxonomy

### *Camptoptera ayezae* Anwar & Zeya sp. nov. (Fig. 1)

Type material. Holotype, ♀ (on slide under 4 coverslips, slide No. MYM.187), INDIA: KARNATAKA: Bengaluru, Jarakabande Kaval, 16.09.2014 (MT), Coll. K Veenakumari. (ZDAMU).

#### Diagnosis

*Camptoptera ayezae* Anwar & Zeya sp. nov. is a remarkable species. The features that make it distinct in the genus are: scape with longitudinal striations, thorax with heavily reticulate sculpture, propodeum with submedian carinae reaching to metanotum and, petiole smooth with lateral lamallae. None of the species from Oriental and Palaearctic region have such a heavy reticulation on thorax.

#### Description

Female. Holotype. Body length, 402 µm. Body dark brown. Antenna dark brown. Wings infumate. Legs, including coxae, largely dark brown except protibia and tarsi lighter.

Head (Fig. 1A). Head, in frontal view, 1.4 times as broad as high; transverse trabecula not divided in middle; area above transverse trabecula and face frontal with reticulate sculptures; occiput with area above transverse suture with transverse striations and, the area below it with longitudinal striations. Mandibles pointed, crossing each other medially. Antenna (Fig. 1B) with scape 4 times as long as broad and, with longitudinal striations; pedicel 1.7 times as long as broad, longer than all funicular segments individually except F3 and, with longitudinal striations; funicle 7-segmented; F3 the longest, F2 ring-like segment; clava 2.8 times as long as broad, longer than F5-F7 combined, with 4 mps.

Mesosoma (Fig. 1D). Mesosoma longer than gaster; mesoscutum with transverse, polygonal reticulations, prosternum pointed apically (Fig. 1E); notaular lines extending to anterior margin of scutellum; scutellum 0.3 times frenum length; scutellum and axilla with reticulate sculpture; axillar seta long; frenum with reticulate sculpture (Fig. 1D); propodeum with submeadian carina reaching to metanotum. Fore wing 17 times as long as broad, disc medially with a row of setae in apical half (Fig. 1C); longest marginal seta 7 times as long as maximum wing width. Hind wing 31 times as long as broad; longest marginal seta 9 times as long as maximum wing width (Fig. 1C).

Metasoma (Fig. 1F). Petiole 1.5 times as long as broad, with a pair of lateral lamellae. Ovipositor, 0.5 times metatibia.

Measurements (holotype slide, µm): head width:height, 140:98; antennal segments length:width-radicle, 8:5; scape, 73:18; pedicel, 33:20; F1, 30:8; F2, 3:8; F3, 35:6; F4, 25:8; F5, 23:10; F6, 25:11; F7, 25:14; clava, 85:30; mesosoma, 150; mesoscutum, 40; scutellum, 15; frenum, 53; metanotum, 8; propodeum, 20; fore wing length:width, 435:25; longest marginal seta, 165; hind wing length:width, 400:13; longest marginal seta, 123; protibia, 95; mesotibia, 138; metatibia, 143; petiole length:width, 30:20; gaster, 100; ovipositor, 75.

Male: Unknown.

Host: Unknown.

Distribution: India: Karnataka.

Etymology: The species name is arbitrary combination of letters and may be taken as a noun in apposition.

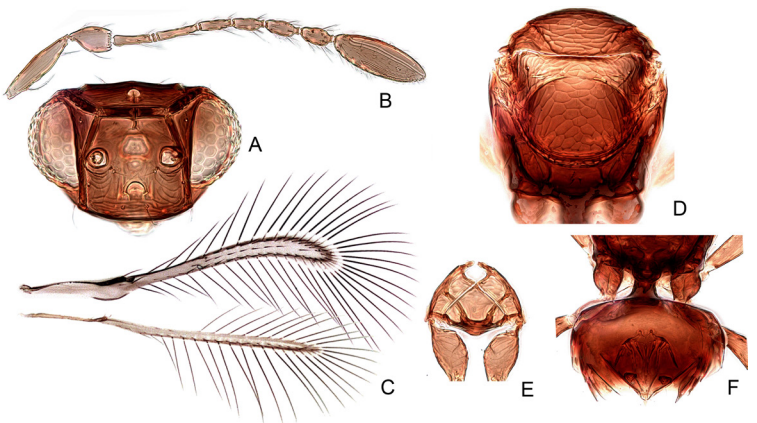


Fig. 1. *Camptoptera ayezae* Anwar & Zeya sp. nov., female, holotype. A, head, frontal, B. antenna, C. wings, D. mesosoma, E. prosternum, F. metasoma.

### *Camptoptera sadhui* Anwar & Zeya sp. nov. (Fig. 2)

Type material. Holotype, ♀ (on slide under 4 coverslips, slide No. MYM.187), INDIA: KARNATAKA: Bengaluru, NBAIR, 1 female, 26.06.2012 (YPT), Coll. K Veenakumari. Paratype, ♀ (on slide under 1 coverslip, slide No. MYM.668), INDIA: KERALA: Alappuzha, Kayamkulam, 17.01.2012 (SN), Coll. F.R. Khan.

### Diagnosis

*Camptoptera sadhui* Anwar & Zeya sp. nov. is a distinct species and is characterized by the antenna with F1, longest, mesoscutum and frenum with polygonal reticulations, propodeum with submedian carina reaching upto metanotum and, petiole with long lateral hair-like lamella. The only other described species of *Camptoptera* from Oriental and Palaearctic regions with longest F1 are *C. assamensis* Rehmat & Anis and *C. poptera* Triapitsyn but both differs from *C. sadhui* as follows: In *C. sadhui* mesoscutum with transverse reticulations, frenum with reticulate sculpture and lateral lamellae long hair-like (in *C. assamensis*: mesoscutum with polygonal reticulate sculpture, frenum with longitudinally parallel reticulate sculpture and, petiole with lateral projections) (in *C. poptera*: mesoscutum and scutellum reticulate, sculpture cells on frenum of scutellum finer than on midlobe of mesoscutum and, petiole with spine-like lateral lamella).

### Description

Female. Holotype. Body length, 270 µm. Body dark brown. Antenna pale brown. Wings hyaline. Legs, including coxae, pale yellow.

Head (Fig. 2A). Head, in frontal view, 1.3 times as broad as high; transverse trabecula not divided in middle; area between transverse trabecula and face frontal with reticulate sculptures. Mandibles pointed, crossing each other medially. Antenna

(Fig. 2B) with scape 2 times as long as broad and, with longitudinal striations; pedicel 1.2 times as long as broad, shorter than all funicular segments individually and, with longitudinal striations; funicle 7-segmented; F1 the longest; clava 3.9 times as long as broad, longer than F5-F7 combined, with 4 mps.

Mesosoma (Fig. 2E). Mesosoma longer than gaster; mesoscutum with transverse reticulations; notaular lines extending to anterior margin of scutellum; scutellum 0.2 times frenum length; scutellum and axilla with reticulate sculpture; frenum with reticulate sculpture (Fig. 2E); propodeum with submeadian carina reaching to metanotum. Fore wing 13 times as long as broad, disc medially with a row of setae in apical half (Fig. 2C); longest marginal seta 5 times as long as maximum wing width. Hind wing 31 times as long as broad; longest marginal seta 10 times as long as maximum wing width (Fig. 2C).

Metasoma (Fig. 2D). Petiole 1.9 times as long as broad, with a pair of long lateral lamellae. Ovipositor, 0.8 times metatibia.

Measurements (holotype slide, µm): head width:height, 113:88; antennal segments length:width-radicle, 5:5; scape, 30:15; pedicel, 18:15; F1, 35:8; F2, 3:5; F3, 28:8; F4, 23:8; F5, 21:8; F6, 23:10; F7, 23:13; clava, 78:30; mesosoma, 138; mesoscutum, 23; scutellum, 9; frenum, 50; metanotum, 4; propodeum, 43; fore wing length:width, 330:25; longest marginal seta, 125; hind wing length:width, 305:10; longest marginal seta, 100; protibia, 75; mesotibia, 105; metatibia, 100; petiole length:width, 28:15; gaster, 113; ovipositor, 75.

Male: Unknown.

Host: Unknown.

Distribution: India: Karnataka, Kerala.

Etymology: The species is named after Professor (Retd.) D. N. Sadhu, Department of Zoology, Vinoba Bhave University, Hazaribag, Jharkhand, India for his excellent contribution in teaching and research.

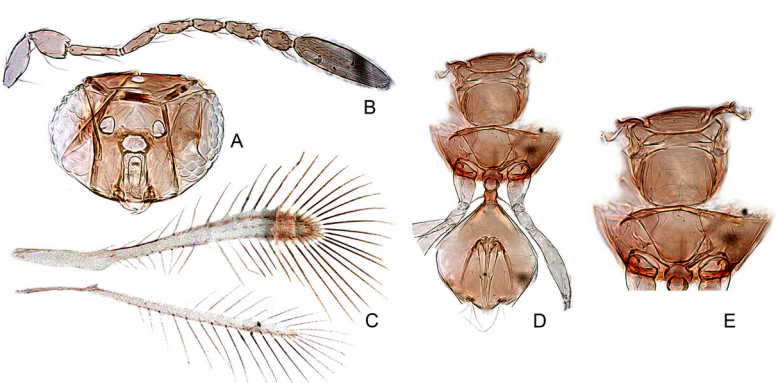


Fig. 2. *Camptoptera sadhui* Anwar & Zeya sp. nov., female, holotype. A. head, frontal, B. antenna, C. wings, D. mesosoma with gaster and petiole, E. mesosoma enlarged.

## ACKNOWLEDGEMENTS

We express our sincere gratitude to Dr. Mohammad Hayat, (ZDAMU) for suggestions and criticism. We are grateful to Dr. K. Veenakumari, formerly of the National Bureau of Agricultural Insect Resources, Bengaluru (NBAIR), for donating the specimens for study. The senior author 'PTA' gratefully acknowledges the Council of Scientific & Industrial Research (CSIR), New Delhi, for providing financial support in the form of "Research Associateship". Z. Ahmad and H. A. Ghramh appreciate the support of the Research Center of Advanced Materials Science, King Khalid University, Abha, Saudi Arabia for supporting the research through research number RCAMS/KKU/22.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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*J. Entomol. Res. Soc., 24(3): 353-373, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2286* *Online ISSN:2651-3579*

# Larvicidal and Growth Inhibitory Activities of *Ageratum houstonianum* (Mill, 1768) Against the Dengue Vector *Aedes aegypti* (Linnaeus, 1762)

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## ABSTRACT

In the present work, *Ageratum houstonianum* leaf acetone extract was tested for the larvicidal and growth inhibitory activity against the third instar larvae of the Dengue vector, *Aedes aegypti.* The extract showed larvicidal activity with $LC_{50}$ and $LC_{90}$ values of 204.79 and 277.57 mg/L, respectively. The larval mortality was increased during subsequent days of treatment. The extract adversely affected larval development, causing a significant reduction in both the formation of fourth instar larvae and pupae. Also, the extract increased the larval duration of *Ae. aegypti* third instar larvae indicating growth inhibitory activities of the extract. The results showed dose-dependent effects of the *A. houstonianum* leaf acetone extract; treatment at higher concentrations inhibited the growth and reduced the viability. This resulted in a decrease in the larval growth index. GC-MS analysis revealed the presence of precocene I and precocene II along with many other components which affect the survival and growth of the insects adversely. This explores the potential of *A. houstonianum* in the management of *Ae. aegypti.*

*Keywords: Aedes aegypti, Ageratum houstonianum,* larvicidal activity, growth and development.

Shah, V.K. & Gupta, K.K. (2022). Larvicidal and growth inhibitory activities of *Ageratum houstonianum* (Mill, 1768) against the dengue vector *Aedes aegypti* (Linnaeus, 1762). *Journal of the Entomological Research Society*, 24(3), 353-373.
*Received: May 07, 2022* *Accepted: October 17, 2022*

## INTRODUCTION

Day-biting mosquito, *Aedes aegypti* Linnaeus (Diptera: Culicidae) is a primary vector of dengue, chikungunya, yellow fever, and Zika viruses (Van den Hurk et al, 2012; Dutra et al, 2016; Munusamy, Appadurai, Kuppusamy, Michael, & Savarimuthu, 2016; Thangamani, Huang, Hart, Guzman, & Tesh, 2016; Liu et al, 2020). Transmission of these disease pathogens to human by mosquitoes threaten over 80% of the world's population and is a major global concern (Golding et al, 2015; Leta et al, 2018). In the last few decades, global climate change coupled with environmental conditions such as high relative humidity and rainfall favoured extensive mosquito breeding and outbreak of many mosquito-borne diseases in tropical and subtropical countries (Kumar, Ammani, Jobina, Subhaswaraj, & Siddhardha, 2017). The incidence of dengue has increased dramatically; dengue alone accounted for an estimated 390 million infections annually worldwide of which 96 million people manifested clinical cases (Bhatt et al, 2013). According to the World Health Organization (WHO), the number of dengue cases increased over eight-fold in the last two decades across the globe. Dengue is also considered one of the primary causes of hospital admission (Ahmed & Khan, 2021). In India, the last two decades witnessed repeated outbreaks, significant geographical spread, and almost an eleven-time increase in the number of dengue cases (Prajapati, Singh, Jain, Srivastava, & Prajapati, 2022).

In the absence of an effective vaccine against most mosquito-borne diseases, the main approach to tackle the mosquito-borne diseases is vector control and vector management. Chemical insecticides are widely used to target the larval stages of the vector (Costa, Naspi, Lucia, & Masuh, 2017; Martianasari & Hamid, 2019). However, the constant use of synthetic insecticides affected non-target organisms and the environment by polluting soil, air, and water. Besides, resistance development to the insecticides in insects makes them disadvantageous (Gayathri & Murthy, 2006; Govindarajan, Sivakumar, Rajeswari, & Yogalakshmi, 2012; Saavedra, Romanelli & Duchowicz, 2018). Integrated vector management (IVM) inspires the ideal use of resources for effective, cheap, and ecologically sustainable vector control (Beier et al, 2008).

Botanicals provide an eco-friendly and sustainable approach to managing the mosquito population (Ruiz-Guerrero, Rodríguez-Pérez, & Norzagaray-Campos, 2015; Pavela, Maggi, Iannarelli, & Benelli, 2019). Plant-based chemicals are species-specific, environmentally safe, less toxic to humans and non-target organisms; and have a fewer possibility of resistance development in insects (Isman, 2000; Sharma, Mohan, & Srivastava, 2006; Demirak & Canpolat, 2022). Earlier reports have confirmed the larvicidal activities of several plant extracts. For instance, Macêdo et al (1997) reported the activities of ethanol extracts of 83 plant species against *Aedes fluviatilis* and found that the extracts from *Tagetes minuta* and *Eclipta paniculata* had high larvicidal potential. The methanolic extract of *Chromolaena odorata* leaves was reported to have larvicidal activity against late instar larvae of *Anopheles stephensi, Culex quinquefasciatus* and *Ae. aegypti* (Sukhthankar, Kumar, Godinho, & Kumar, 2014); also, the leaf extract of *Ambrosia arborescens* was found effective against third instar

larvae of *Ae. aegypti* (Morejón et al, 2018). In addition, secondary metabolites present in the plants can provide natural candidates for developing novel larvicides, repellents, oviposition deterrents, and growth inhibitors for the control of insect vectors (Cavalcanti et al, 2004; Ríos, Stashenko, & Duque, 2017; Bekele, 2018; de Souza Wuillda et al, 2019). The larvicidal properties of different classes of compounds of natural origin against malaria mosquitoes were highlighted by Milugo et al (2021). Recent research on plant-extract derived nanoparticles has shown a great promise in the management of vectors. Nanoparticles of aqueous extracts of *Ambrosia arborescens* (Morejón et al, 2018) and leaf extract of *Citrus medica*, *Tagetes lemmonii* and *Tarenna asiatica* (Chandhirasekar et al, 2021) against *Ae. aegypti* showed larvicidal activity. The *Ageratum houstonianum* Mill. (Family: Asteraceae) is an invasive herbaceous weed commonly known as 'Floss flower' or 'blue mink' (BioNet-EAFRINET, 2016). The plant is native to Mexico and Central America (Njateng et al, 2010) and is widely distributed in tropical, subtropical and temperate climatic zones (Bhellum, 2020; Chandraker et al, 2020) including India and Asian countries (Ambasta, 1988; Sharma, 1987). Different species of *Ageratum* are used for relieving sore throats, fever, rheumatism, skin and stomach infections in the aboriginal systems of medicine (Sharma & Sharma, 1995; Andrade-Cetto, 2009). It is also used to cure wounds and burns (Durodola, 1977) and as an anti-inflammatory agent to relieve swelling and pain in the throat (Ming, 1999). Moreover, it is a curative plant reported to possess antifungal (Pandey et al, 1983), antimicrobial (Kurade et al, 2010) and antioxidant activities (Tennyson et al, 2012). Insecticidal properties (Bowers et al, 1976; Ravindran, Samuel, Alex, & William, 2012) and insecticidal compounds (Renuga & Sahayaraj, 2009) including precocenes were reported in this plant (Kumar, 2014). The crude extract of *A. houstonianum* had shown the mosquitocidal properties (Boussaada et al, 2008; Sakthivadivel & Daniel, 2008; Pavela, 2009; Senthilkumar, Varma, & Gurusubramanian, 2009; Elango et al, 2010) including adulticidal (Ravindran et al, 2012), larvicidal (Tennyson, Ravindran, Eapen, & William, 2015a), ovicidal (Tennyson, Ravindran, Eapen, & William, 2015b); the extract also showed repellent (Tennyson, Ravindran, Eapen, & William, 2012a) and oviposition deterrent activities (Tennyson, Ravindran, Eapen, & William, 2012b). The phytochemical composition of *A. houstonianum* showed the presence of chromenes, precocene I and II, which have been described for their anti-JH activity (Haunerland & Bowers, 1985; Binder, Bowers, & Evans, 1991; Lu et al, 2014).

Most of the studies on *A. houstonianum* were focused on cidal activity and lethal effects on mosquitoes (Sharma et al, 2006; Arivoli & Tennyson, 2011; Ravindran et al, 2012; Tennyson et al, 2015a). However, the biological activities of acetone extract of *A. houstonianum* have not been specified against *Ae. aegypti*. The type of solvent used for extraction affects the larvicidal activity (Zhang, Lin, & Ye, 2018). Growth and development are important components of insect life; aberration of these can hamper the insect population. With this aim, larvicidal and growth inhibitory efficacy of the *A. houstonianum* leaf acetone extract against third instar larvae of *Ae. aegypti* were undertaken in the present research work. The phytoconstituents of the *A. houstonianum* leaf acetone crude extract were determined using GC-MS analysis. The larvicidal and

growth inhibitory studies were correlated with the compounds identified in the GC-MS profile of the extract. The present studies are important in the search for effective affordable natural products which can be used in the IVM program of *Ae. aegypti.*

## MATERIALS AND METHODS

### Rearing and maintenance of *Aedes aegypti* culture

A stock culture of *Ae. aegypti* was procured from 'International Center for Genetic Engineering and Biotechnology (ICGEB)', New Delhi, India, and maintained in an insectary under optimum conditions of temperature 28±1°C, relative humidity 80±5%, and photoperiod 14L:10D, to obtain insects of sustained quality throughout the research work (Shazad et al, 2018). Each larval stage was reared in enamel coated bowl or tray containing dechlorinated water according to the protocol laid by WHO (WHO, 2005). Larval diet was composed of dog biscuits and yeast in a 3:1 ratio. The pupae were separated, transferred into enamel bowls, and kept in the mosquito cages. Adults were fed upon raisins and adult female mosquitoes were blood-fed after 2 days of emergence on albino rats for maturation of eggs.

### Plant collection and preparation of acetone extract

The leaves of the *Ageratum houstonianum* were collected during the month of March, 2021 from the fields adjoining the Delhi state, India (geographic coordinates 28°49'14.97"N and 76°46.3776"E). The leaves were washed thoroughly, shade dried at room temperature for a week, and mechanically ground to make a fine powder. The leaves powder was extracted continuously in acetone, using the 'Soxhlet extraction apparatus' at 45°C for 24 h. Subsequently, the extract was filtered through Whatman filter paper No. 1 and finally, concentrated using a 'Rotavapor vacuum evaporator (Buchi)'. The percentage of extraction was calculated by using the Equation No. 1,

$$\text{Percent extraction} = \frac{\text{Weight of the extract}}{\text{Weight of the plant material}} \times 100 \quad \text{.....Eqn. 1}$$

The 10% stock solution of the extract was prepared by mixing 1 part of the extract in 9 parts dimethyl sulfoxide (DMSO) and stored at 4°C for further use.

### Larvicidal bioassay

The larvicidal efficacy of *A. houstonianum* leaf acetone extract was assessed by performing a bioassay against laboratory-bred early third instar larvae of *Ae. aegypti*. The third larval stage was exposed to crude *A. houstonianum* leaf acetone extract of concentrations 50, 100, 150, 200, 250 and 300 mg/L for 24 h using the standard WHO protocol (WHO, 2005). Twenty-five newly emerged third instar larvae of *Ae. aegypti* were placed in enamel-coated bowls containing the 1 ml of test samples diluted in the 249 ml of dechlorinated water. In control, 1ml of DMSO was added to 249 ml of dechlorinated water. The larval mortality was observed after 24 h of the exposure period. The larvae were considered dead when no movement was shown by the larva

on gentle probing with a needle. Also, all moribund larvae were considered as dead. The experiments were repeated four times with each tested concentration and control.

**Effects of *A. houstonianum* leaf acetone extract on growth of *Ae. aegypti***

The effect of *A. houstonianum* leaf acetone extract was studied on the survival, growth and development of *Ae. aegypti.* The third instar larvae were treated with 50, 100, 150 and 200 mg/L of *A. houstonianum* leaf acetone extract for 72 h and then transferred to freshwater. The number of larvae found dead, and the number of fourth instar larvae and pupae formed were recorded daily. The data was analyzed to calculate day-wise mortality, time taken by third instar larva to form a pupa, and the percent of third instar larvae moulted into fourth instar larvae and formed pupae. The impact of *A. houstonianum* leaf acetone extract on the development of the third instar larva till pupa formation was measured by considering the larval growth index i.e., ratio of percent pupae formation to the average time taken by the third instar larva to form a pupa. All the experiments were replicated four times; 25 third instar larvae were taken in each replicate.

**GC-MS analysis of the *A. houstonianum* leaf acetone extract**

The phytochemicals present in the acetone extract of *A. houstonianum* leaves were analyzed through gas chromatography and mass spectroscopy (GC-MS) (Ezhilan & Neelamegam, 2012). The concentrated extract of *A. houstonianum* was dissolved in acetone and injected into the 'Gas chromatography unit (Shimadzu GC-MS QP2010)'. The injector temperature was maintained at 250ºC. The detector used was a flame ionization detector which was maintained at a temperature of 280ºC. The pressure of the carrier gas, nitrogen, was kept at 10 psi. The oven temperature was set from 60ºC to 280ºC with a gradual increment of 10ºC per minute. The injected extracts were eluted in the DB-5 MS column of 30 m long and 0.25 mm inner diameter and the eluted constituents were detected by a flame ionization detector. The GC chromatogram was recorded and the compounds were identified by comparing the data with the existing software libraries like WILEY08, NIST08, and NIST08s (Hübschmann, 2015).

**Statistical analysis**

The mortality in the experimental tests was corrected by using Abbott's formula presented in Equation No. 2 (Abbott, 1925).

Corrected percent mortality:

$$= \frac{\%\ \text{treated mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100 \quad \text{.....Eqn. 2}$$

The survival data obtained from the bioassay experiment was subjected to regression analysis. The lethal concentration $LC_{90,}$ median lethal concentration $LC_{50,}$ and values of sublethal concentrations ($LC_{10}$ and $LC_{30}$) with 95% fiducial limits were calculated in bioassay. All quantitative data were analyzed using descriptive statistics in SPSS version 19.0 software (SPSS, Chicago, IL, USA) and MS excel 2016. One-way ANOVA followed by the Tukey's post hoc test was used to determine and identify statistically significant

differences between groups at a *p-value* <0.05. Results with the value of *p*<0.05 were considered to be statistically significant (Finney, 1971; Fisher, 1992).

## RESULTS

Extraction of 200 g of the *A. houstonianum* leaves powder was done with 1L of acetone in the Soxhlet apparatus for 24 h, 4-5 cycles per h, at 45°C. This process yielded 17.3 g i.e., 8.65% crude extract.

Distinct effects of *A. houstonianum* leaf acetone extract were reported on the survival of the third instar larva of *Ae. aegypti.* The results indicate that the extract was toxic to the third instar larva and caused larval mortality within 24 h. The regression analysis revealed the toxicity of the extract in a dose-dependent manner (Fig. 1). The $LC_{50}$ and $LC_{90}$ values were 204.79 and 277.57 mg/L, respectively. The values of sublethal concentrations of *A. houstonianum* leaf acetone extract i.e., $LC_{10}$ and $LC_{30}$ were 151.09 and 180.83 mg/L, respectively (Table 1). Treatment of early third instar larvae to the extract of concentrations 50 and 100 mg/L did not show significant mortality after 24 h of exposure.

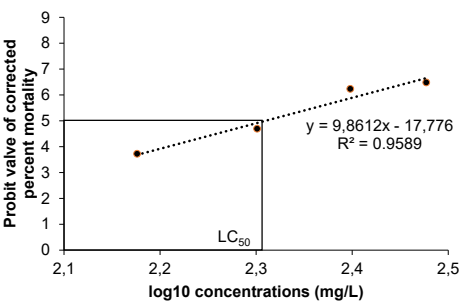



Fig. 1. The response of third instar larvae of *Ae. aegypti* to the acetone extract of *A. houstonianum* leaves. The graph represents regression line of relationship between log concentrations of extract and probit value of corrected per cent mortality.

Table 1. Lethal and sublethal concentrations of the *A. houstonianum* leaf acetone extract against third instar larvae of *Ae. aegypti* after 24 hours of exposure.

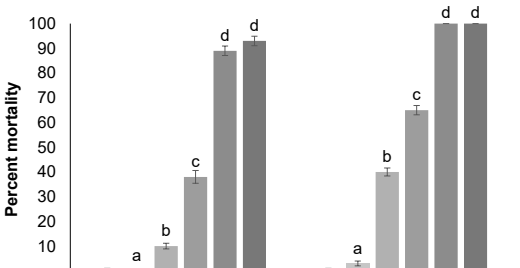
| LC levels | Estimated LC values (mg /L) | 95% Confidence Limits for Concentrations (mg /L) | | Regression Equation | $R^2$ (Regression coefficient) | $\chi^2$ (Chi-square value) | *p -value* |
|---|---|---|---|---|---|---|---|
| | | Lower Limit | Upper Limit | | | | |
| $LC_{10}$ | 151.09 | 135.12 | 168.96 | y = 9.8612x -17.776 | 0.958 | 0.561 | 0.021 |
| $LC_{30}$ | 180.83 | 161.71 | 202.21 | | | | |
| $LC_{50}$ | 204.79 | 183.14 | 229.00 | | | | |
| $LC_{90}$ | 277.57 | 248.23 | 310.39 | | | | |

Quantitative data were analyzed using descriptive statistics in SPSS version 19.0 software (SPSS, Chicago, IL, USA) and MS excel 2016.

Average of four replicates, 25 third instar larvae per replicate.

The null hypothesis is rejected: F value > F critical, and *p* (≤0.001) < α=0.05; LC = Lethal Concentrations; LC10, LC30, LC50, and LC90 = Lethal Concentrations for 10%, 30%, 50% and 90% mortality with 95% confidence limits.

Continued exposure to *Ae. aegypti* third instar larvae to the *A. houstonianum* extract caused an increase in mortality on subsequent days; the larvicidal efficacy of the extract increased as the exposure time increased (Fig. 2). Treatment with the extract of concentrations 250 and 300 mg/L caused a hundred percent larval mortality within 2-3 days of exposure (Table 2). Also, the total mortality of third instar larvae was increased when treated with extract of concentrations 150 and 200 mg/L. The results were significantly different in comparison to the control. The extract of concentrations 50 and 100 mg/L was not effective in causing mortality. No significant increase in mortality was observed at these concentrations during subsequent days of exposure (Fig. 2, Table 2).




| | % mortality after 24 hr | Total % mortality |
|---|---|---|
| 50 mg/L | 0 | 0 |
| 100 mg/L | 0 | 3 |
| 150mg/L | 10 | 40 |
| 200 mg/L | 38 | 65 |
| 250 mg/L | 89 | 100 |
| 300 mg/L | 93 | 100 |

Fig. 2. Comparison of percent larval mortality after 24 h of treatment and total mortality of third instar larvae of Ae. aegypti exposed to the A. houstonianum leaf acetone extract for three days. The same letters on the bar graph of one category are not significantly different at $p<0.05$ (ANOVA followed by Tukey's test).

Table 2. Cumulative larvicidal activity of the *A. houstonianum* leaf acetone extracts on third instar larvae of *Ae. aegypti.*

| Concentration of the extract (mg/L) | Day-wise cumulative corrected percent mortality* (Mean ± S.E.) | | | |
|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 |
| **50** | $0^a \pm 0$ | $0^a \pm 0$ | $0^a \pm 0$ | $0^a \pm 0$ |
| **100** | $0^a \pm 0$ | $0^a \pm 0$ | $3^a \pm 1$ | $3^a \pm 1$ |
| **150** | $10^b \pm 1.15$ | $17^b \pm 2.52$ | $35^b \pm 1.91$ | $40^b \pm 1.63$ |
| **200** | $38^c \pm 2.58$ | $49^c \pm 1.91$ | $60^c \pm 1.63$ | $65^c \pm 1.91$ |
| **250** | $89^d \pm 1.91$ | $97^d \pm 1$ | $100^d \pm 0$ | - |
| **300** | $93^d \pm 1.91$ | $100^d \pm 0$ | - | - |
| **df** | 5,18 | | 4,15 | 3,12 |
| **F critical value** | 2.773 | | 3.056 | 3.490 |
| **F value** | 727.617 | 1153.182 | 1192.023 | 530.545 |
| ***p value*** | $p \leq 0.001$ | | | |

df-degree of freedom, *Average of four replicates, 25 third instar larvae per replicate. Mortality in the test was corrected by Abbott's formula. Means followed by the same letters in a column are not significantly different at $p<0.05$ (ANOVA followed by Tukey's test). The null hypothesis is rejected: F value > F critical, and $p$ (≤0.001) < α=0.05.

Effect of *A. houstonianum* leaf acetone extract on the growth of third instar larvae of *Ae. aegypti* was evaluated by assessing the day-wise formation of fourth instar larvae and pupae from the treated larvae, time taken by the treated larva to form a pupa, and larval growth index. The results indicate continuous exposure of third instar larvae of *Ae. aegypti* to *A. houstonianum* leaf acetone extract caused a delay in fourth instar larva formation. In control, 86% of the third instar larvae were moulted to fourth-instar larvae within two days and almost all of them molted within 4 days. On the other hand, when treated with the extract of concentrations 50 and 100 mg/L, less than 50% of the third instar larvae moulted to the fourth instar larvae in two days; it was increased during subsequent days. The percent of larvae moulted to the fourth stage remained low throughout the treatments and exceeded for more than six days in the treatments with the extract of concentrations 150 and 200 mg/L (Table 3).

Table 3. Effect of *A. houstonianum* leaf acetone extract on the 4th instar larvae formation from third instar larvae of *Ae. aegypti*.

| Concentration of the extract (mg/L) | Day-wise cumulative percent of fourth larva formation* (Mean ± S.E.) | | | | |
|---|---|---|---|---|---|
| | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** |
| Control | $49^{a} \pm 3.42$ | $86^{a} \pm 2.58$ | $97^{a} \pm 1$ | $100^{a} \pm 0$ | - |
| 50 | $35^{b} \pm 1.91$ | $44^{b} \pm 2.31$ | $66^{b} \pm 1.15$ | $94^{a} \pm 1.15$ | $100^{a} \pm 0$ |
| 100 | $34^{b} \pm 2.58$ | $45^{b} \pm 1$ | $61^{bc} \pm 2.52$ | $85^{b} \pm 3.42$ | $97^{a} \pm 1$ |
| 150 | $39^{ab} \pm 3.42$ | $47^{b} \pm 2.52$ | $55^{c} \pm 1$ | $57^{c} \pm 1$ | $60^{b} \pm 1.63$ |
| 200 | $17^{c} \pm 3$ | $23^{c} \pm 1.91$ | $29^{d} \pm 1.91$ | $35^{d} \pm 1.91$ | - |
| **df** | 4,15 | | | | 2,9 |
| **F critical value** | 3.056 | | | | 4.256 |
| **F value** | 15.727 | 113.587 | 223.050 | 212.745 | 406.091 |
| ***p value*** | $p \leq 0.001$ | | | | |

df-degree of freedom, *Average of four replicates, 25 third instar larvae per replicate. Means followed by the same letters in a column are not significantly different at $p<0.05$ (ANOVA followed by Tukey's test). The null hypothesis is rejected: F value > F critical, and $p$ (≤0.001) < α=0.05.

Efficacy of the *A. houstonianum* leaf acetone extract was also assessed on pupae formation from treated third instar larvae of *Ae. aegypti*. In control, it was seen that pupae formation was completed within 5 days from the third instar larvae (Table 4). Also, 50% of pupal formation took place within 3 days. On the other hand, in the larvae treated with the extract of concentration 50 mg/L, the pupal formation was delayed; only 11% of pupae formed in three days. The duration for pupa formation was extended up to six days from the day of treatment of the third instar larvae. In the treatment with the extract of concentration 100 mg/L, treated third instar larvae took up to seven days to form pupae. The number of pupae formed on three days was less. The delay in the pupae formation was further increased in the treatments with the extract of concentrations 150 and 200 mg/L (Table 4).

Table 4. Effect *of A. houstonianum* leaf acetone extract on the pupa formation from third instar larvae of *Ae. aegypti*.

| Concentration of the extract (mg/L) | Day-wise cumulative percent of pupa formation* (Mean ± S.E.) | | | | |
|---|---|---|---|---|---|
| | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 7** |
| Control | $50^{a}$ ± 3.46 | $76^{a}$ ± 1.63 | $100^{a}$ ± 0 | - | - |
| 50 | $11^{b}$ ± 1.91 | $42^{b}$ ± 2.58 | $93^{b}$ ± 1 | $98^{a}$ ± 1.15 | - |
| 100 | $9^{b}$ ± 1.91 | $23^{c}$ ± 1.91 | $58^{c}$ ± 1.15 | $76^{b}$ ± 1.63 | $90^{b}$ ± 2 |
| 150 | $2^{b}$ ± 1.15 | $8^{d}$ ± 1.63 | $26^{d}$ ± 1.15 | $41^{c}$ ± 1.91 | $57^{c}$ ± 3 |
| 200 | $4^{b}$ ± 1.63 | $10^{d}$ ± 1.15 | $14^{e}$ ± 2 | $22^{d}$ ± 2 | $34^{d}$ ± 1.15 |
| **df** | 4,15 | | | 3,12 | 2,9 |
| **F critical value** | 3.056 | | | 3.490 | 4.256 |
| **F value** | 83.936 | 233.588 | 969.913 | 400.314 | 165.837 |
| ***p value*** | $p \leq 0.001$ | | | | |

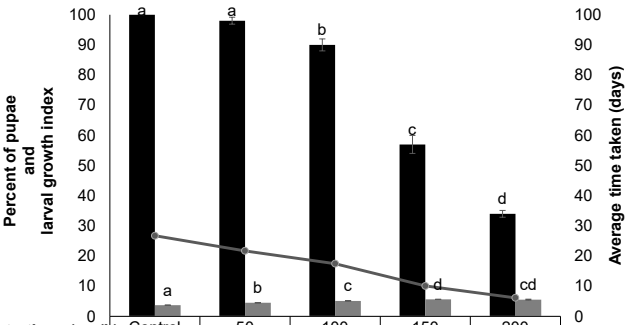
First pupa was formed on day 3. df-degree of freedom, *Average of four replicates, 25 third instar larvae per replicate. Means followed by the same letters in a column on a particular day are not significantly different at $p<0.05$ (ANOVA followed by Tukey's test). The null hypothesis is rejected: F value > F critical, and $p$ (≤0.001) < α=0.05.

It was reported that *A. houstonianum* leaf acetone extract affected the development period of treated third instar larvae of *Ae. aegypti*. Consequently, the larval duration, as measured by the average time taken by the third instar larva to form a pupa, increased in a dose-dependent manner (Fig. 3). In control, the third instar larva took on an average of 3.74 days to complete larval development and form a pupa. However, the larval duration of the treated instars increased to 4.51 and 5.15 days in the treatments with the extract of concentrations 50 and 100 mg/L, respectively. In the treatments with the extract of concentrations 150 and 200 mg/L, the effect was most conspicuous, the larval period of the treated third instar increased to nearly six days which was almost 1.5 times more than the larval period of the third larva in the control (Fig. 3).

The data presented in Figure 3 also indicate that there was a decrease in the larval growth index with the increase in the concentration of extract in the treatments. In control, the larval growth index was 26.75. In treated larvae, the larval growth index continuously decreased with an increase in the extract concentrations; it declined to 6.17 in the treatments with the extract of concentration 200 mg/L. The difference in the results was statistically significant ($p<0.001$). It was also observed that a decrease in the growth index of the larva was due to both decrease in the percentage of total pupae formation and an increase in the average time taken by the third instar larva to form a pupa.

GC-MS analysis of the *A. houstonianum* leaf acetone extract revealed 90 peaks, each representing a specific compound (Fig. 4). Some of the important chemical compounds which affect the life processes of insects are tabulated in Table 5. These include Precocene I, Precocene II, 1h-inden-1-one, Trans ß-caryophyllene, trans-Z-alpha-Bisabolene epoxide, Phytol, n-hexadecanoic acid, α-Linolenic acid, ɣ-Sitosterol, ß-Stigmasterol, Neophytadiene, 2-Pentadecanone, Squalene, Oxymorphone or Morphinan-6-one,

Vitamin E, α-Copaene, ß-copaene, α-farnesene, Cubebol and τ-Cadinol. The biological activities of these compounds in various life processes of insects are listed in Table 5.




| Concentrations (mg/L) | Control | 50 | 100 | 150 | 200 |
|---|---|---|---|---|---|
| percent pupae formed | 100 | 98 | 90 | 57 | 34 |
| Average time for pupa formation | 3,74 | 4,51 | 5,15 | 5,64 | 5,53 |
| Larval Growth index | 26,75 | 21,73 | 17,47 | 10,09 | 6,17 |

Fig. 3. Influence of the acetone extract of *A. houstonianum* leaves on the pupal formation, larval duration, and larval growth index.

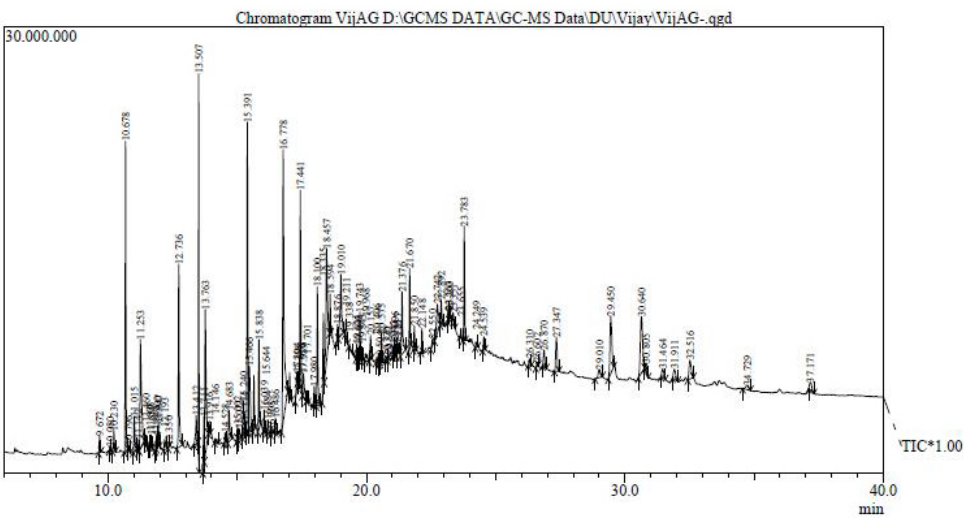



Fig. 4. GC MS chromatogram of the acetone extract of the leaves of *A. houstonianum*, precocene I (arrow) & precocene II (circle) peaks.

Table 5. GC MS analysis of *A houstonianum* leaf acetone extract. Some of the major components present in the extract and their biological activities are represented in the Table.

| S. No. | R/T* | Peak Area (%) | Name of the Compound | Biological activities | References |
|---|---|---|---|---|---|
| 1 | 11.253 | 4.29 | Precocene I | suppress metamorphosis ovarian activation, aggression and alters sterility signal production | Amsalem, Teal, Grozinger, & Hefetz, 2014; Pener, Orshan, & De Wilde, 1978) |
| 2. | 13.507 | 4.40 | Precocene II | suppress metamorphosis, anti-allatal activities, induce precocious metamorphosis | (Bowers & Feldlaufer, 1982; Ohta, Kuhr, & Bowers, 1977; Pener et al, 1978) |
| 3. | 13.763 | 4.76 | 1h-inden-1-one | antimicrobial, anticancer, anti-inflammatory | (Rovnyak, Millonig, Schwartz, & Shu, 1982; Velaparthi et al, 2008) |
| 4. | 10.678 | 5.63 | Trans ß-caryophyllene | weak larvicidal activity | (Dória et al, 2010; Liu & Liu, 2014) |
| 5. | 12.736 | 4.20 | trans-Z-alpha-Bisabolene epoxid℮ | Unknown | |
| 6. | 18.100 | 2.77 | Phytol | antimicrobial, antitumor, anti-teratogenic, antidiabetic, antioxidant, anti-inflammatory, antidepressant, hair fall defense, and antidandruff activities. | (Islam et al, 2015) |
| 7. | 16.778 | 9.35 | n-hexadecanoic acid | anti-inflammatory, antibacterial | (Aparna et al, 2012) |
| 8. | 18.457 | 4.73 | α-Linolenic acid | antitumor activity | (Xu et al, 2021) |
| 9. | 30.640 | 3.41 | γ-Sitosterol | anti-diabetic activity | (Balamurugan, Duraipandiyan & Ignacimuthu, 2011) |
| 10. | 29.450 | 2.84 | ß-Stigmasterol | anti-inflammatory effects, anti-diabetic activity | (Morgan et al, 2021; Wang et al, 2017) |
| 11. | 15.391 | 6.57 | Neophytadiene | anti-inflammatory potential | (Bhardwaj, Sali, Mani, & Vasanthi, 2020) |
| 12. | 15.466 | 2.59 | 2-Pentadecanone | repellent, anticancer activity | (Innocent et al, 2008; Swantara et al, 2019) |
| 13. | 23.783 | 2.22 | Squalene | anticancer, antioxidant, drug carrier, detoxifier, skin hydrating, and emollient activities | (Kim & Karadeniz, 2012) |
| 14. | 18.335 | 1.83 | Oxymorphone or Morphinan-6-one | treating moderate to severe pain, analgesic potency | (Adams, Pieniaszek Jr, Gammaitoni, & Ahdieh, 2005; Prommer, 2006) |
| 15. | 27.347 | 1.29 | Vitamin E | antioxidant activity | (Higgins et al, 2020) |
| 16. | 9.672 | 0.47 | α-Copaene | antileishmanial activity | (Rodrigues et al, 2018) |
| 17. | 10.230 | 0.54 | ß-copaene | anti-inflammatory | (Kadhim, Mohammed, & Hameed, 2016) |
| 18. | 10.796 | 0.10 | α-farnesene | oviposition stimulant | (Yan, Bengtsson, Makranczy & Löfqvist, 2003) |
| 19. | 11.659 | 0.21 | Cubebol | growth inhibition activities, repellent activities, larvicidal activity | (Chen et al, 2001; Gu et al, 2009; Saijo et al, 2013) |
| 20. | 13.412 | 0.85 | τ-Cadinol | antioxidant potential, allelopathic activity | (Abd El-Gawad, El-Amier, & Bonanomi, 2018; Gunes et al, 2021) |

*R/T: retention time

## DISCUSSION AND CONCLUSION

Extraction of *A. houstonianum* leaves with acetone using Soxhlet apparatus yielded 8.65% crude extract. This yield was high in comparison to the sequential extraction method with hexane, ethyl acetate, and methanol which yielded 0.84%, 2.90%, and 1.21% (w/w) (Ravindran et al, 2012). The high yield of the extract may be due to the choice of extraction methods, agitation, time, and nature of the solvent used in the present study. These factors have been shown to influence extract yield (Mohamad, Ali, Ripin & Ahmad, 2013; Andrade et al, 2015; Zhang et al, 2018).

$LC_{50}$ and $LC_{90}$ values of *A. houstonianum* leaf acetone extract against early third instar larvae of *Ae. aegypti* reported in the present work were 204.79 and 277.57 mg/L, respectively. Also, complete larval mortality was recorded at these concentrations during subsequent days of exposure. Larvicidal activities of hexane, ethyl acetate, and methanol crude leaf extracts of *A. houstonianum* against three vector species *viz., An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were described by Tennyson et al (2015a). Our studies showed that the *A. houstonianum* leaf acetone extract was more effective in comparison to these studies. The larvicidal activity differs depending on the species of *Ageratum.* Hussaini et al (2018) reported a moderate toxic effect of methanol and n-hexane extract of *A. conyzoides* leaf on the third-fourth instar larvae of *An. gambiae*; the $LC_{50}$ values for methanol and n-hexane extracts were 423.52 and 627.90 ppm, respectively. The $LC_{50}$ values of the crude methanol, petroleum ether and carbon tetrachloride leaf extracts of *A. conyzoides* against *Cx. quinquefasciatus* were 5105.0, 425.6 and 3139.3 ppm, respectively (Sharma et al, 2006). Pintong et al (2020) reported that none of the six types of crude ethanol extracts obtained from *A. conyzoides* had considerable effects at a concentration of 10 mg/L against early fourth instar larvae of *Ae. aegypti*. Sakthivadivel & Daniel (2008) reported that petroleum ether leaf extract of *A. conyzoides* was effective against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus.*

The studies indicated that the *A. houstonianum* leaf acetone extract had a growth inhibitory effect on fourth instar larvae and pupae development. Therefore, there was an increase in the larval duration of the treated third instar larvae of *Ae. aegypti*. Earlier reports have shown similar trends in growth patterns in response to many plant extracts. For instance, Shazad et al (2018) reported that the ethanol extract of *Ocimum sanctum* prolonged the larval duration of *Ae. aegypti* fourth instar larva. Ferdinand (2014) reported that the larval period of *Spodoptera litura* was prolonged by chloroform and ethanol extracts of both *A. conyzoides* and *Artemesia vulgaris*. Muthukrishnan, Pushpalatha, & Kasthuribhai (1997) reported that the active fractions of *Solanum suratense* ethyl acetate extract extended the larval duration of *Cx. quinquefasciatus*. Zhong et al (2001) have also highlighted that ethyl acetate extract of *Rhododendron molle* flower increased the development duration of *Pieris rapae*. The duration of the larval period of *Tribolium castaneum* was extended by methanol extracts of *Raphanu sraphanistrum* and *Peganum harmala* (Jbilou et al, 2008).

Further, the results showed that larval growth index i.e., the ratio of percent pupae formation to the average time taken by the third instar larva to form a pupa, decreased

in a dose-dependent manner. This is due to both the reduction in pupa formation and the increase in the larval development period of the treated third instar larva. The results are in agreement with da Silva et al (2013), who reported that the n-hexane extract of *Hypericum polyanthemum* inhibited the pupa formation and adult emergence of *Ae. aegypti* at sublethal doses $LC_{10}$ and $LC_{20}$. These studies suggested growth inhibitory activities of the *A. houstonianum* leaf acetone extract on the third instar larvae of *Ae. aegypti*. Jeyabalan et al (2003) described the growth-inhibiting effect of methanol leaf extract of *Pelargonium citrosa* against *An. stephensi*. Consequently, larval and pupal development was completely inhibited by the treatment. It was proposed that the extract contained some compounds which slowed the process of development. The increase in the development period of larva could be related to the disruption of endocrine systems controlling molting, and the synthesis of hormones essential for growth (Lange et al, 1983; Ferdinand, 2014).

Larvicidal and growth inhibitory effects observed in the present study led to the investigation of phytocompounds present in the *A. houstonianum* leaf acetone extract using GC-MS. Our GC-MS chromatogram revealed the presence of 50 components in the crude acetone extract. Chandra et al (1996) found 50 components in the essential oil of *A. houstonianum*. Lu et al (2014) reported a total of 35 components and Hadidy et al (2019) reported 32 components in the essential oil of *A. houstonianum* leaves and flowers, respectively. Further, the GC-MS chromatogram showed the presence of chromene compounds i.e., precocenes; precocene I and precocene II were found most common compounds followed by n-hexadecanoic acid, a pentacyclic triterpene compound and trans-ß-caryophyllene. Precocene I and II were identified as the active components of the essential oil of *A. houstonianum* (Lu et al, 2014). Presence of similar compounds i.e., precocene I, precocene II and β-caryophyllene in the essential oil of *A. houstonianum* was also reported by other researchers (Chandra et al, 1996; Kurade et al, 2010; Lu et al, 2014). Our study also reported the presence of trans-Z-alpha-Bisabolene epoxide, Neophytadiene, 2-Pentadecanone, and Oxymorphone, which were not reported earlier. The difference in components count may be related to the site, habitat, season of plant collection, and organic solvents and techniques used for extractions (Shaalan et al, 2005; Mohamad et al, 2013; Andrade et al, 2015).

Precocenes and cubebol are the most important components of the crude acetone extract considering insect-related activities. The toxicity of the extract to *Ae. aegypti* larvae may be attributed to precocene I and precocene II or synergistic interaction between these components and the other constituents of the extract (da Silva et al, 2013). The results are congruent with studies of Liu & Liu (2014), who reported that precocene I and II exhibited larvicidal activity against the 4$^{th}$ instar larvae of *Ae. albopictus*. Further, cubebol showed growth inhibition activities against *Heterosigma akashiwo* (Saijo et al, 2013) and larvicidal activity against *Ae. aegypti* larvae (Gu et al, 2009). Furthermore, previous studies demonstrated that precocene I and II hinder the juvenile hormone synthesis in several insects. Bowers et al (1976) reported that adults insects treated with precocenes became sterile and juveniles showed precocious metamorphosis and immediate death of premature adults. These compounds may have similar actions on

the dengue vector. Consequently, this can disturb embryonic development, induce premature metamorphosis, decrease the reproductive potential, and affect the insect behavior including the antifeedant and repellent effect (Bowers et al, 1976; Srivastva & Kumar, 1997; Khafagi & Hegazi, 2004; Lu et al, 2014). Moreover, precocene I and II exhibited larvicidal and growth-inhibiting activities against *An. stephensi* (Saxena et al, 1994). Further, the benzopyrans HP1-HP3, the major compounds of *Hypericum polyanthemum*, are structurally similar to precocenes and showed larvicidal and growth-regulating activity against *Ae. aegypti* (da Silva et al, 2013).

The present investigation explored the prospective role of phytochemicals present in the *A. houstonianum* leaf acetone extract as larvicide and growth inhibitor in the management of mosquito, *Ae. aegypti*. Our study suggested that the *A. houstonianum* leaf acetone extract has larvicidal, growth, and developmental disrupting activities against *Ae. aegypti.* The extract showed larvicidal activity against *Ae. aegypti* early third instar larvae; larval mortality was increased with an increase in the concentrations. Moreover, the extract at lower concentrations significantly prolonged the larval duration of the survived third instar larva of *Ae. aegypti*. The GC-MS chromatogram of the *A. houstonianum* leaf acetone extract showed the presence of anti-JH compounds i.e., precocene I and II. Thus, *Ae. aegypti* population could be impeded in the juvenile stages. The results documented showed the potential of *A. houstonianum* leaves as a source of new insecticides for the integrated vector management of *Ae. aegypti*.

## ACKNOWLEDGMENTS

The authors are thankful to the principal, Deshbandhu college for providing the required facilities. Vijay Kumar Shah acknowledge Council of Scientific and Industrial Research India for providing financial assistance to carry out research work.

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*J. Entomol. Res. Soc., 24(3): 375-387, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2295* *Online ISSN:2651-3579*

# Contributions to the Faunistic Knowledge of Chironomidae (Diptera) of Turkey Based on the Adult Males Collected Around Hazar Lake (Elazığ)

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## ABSTRACT

In the present study, adult male specimens from the non-biting midge family (Chironomidae) collected around the Hazar Lake (Elazığ, Türkiye) in 2021 were evaluated taxonomically. We collected a total of 17 species in three subfamilies of Tanypodinae, Orthocladiinae, and Chironominae. Two species *Cryptotendipes pseudotener* (Goetghebuer, 1922), and *Dicrotendipes pallidicornis* (Goetghebuer, 1934) are new faunistic records for Türkiye. 11 species were observed for the first time in the Hazar Lake, although they have been reported previously in Türkiye. These include: *Procladius (Holotanypus) choreus* (Meigen, 1804), *Cricotopus (Cricotopus) bicinctus* (Meigen, 1818), *Cricotopus (Cricotopus) festivellus* (Kieffer, 1906), *Cryptotendipes pseudotener* (Goetghebuer, 1922), *Cryptochironomus (Cryptochironomus) rostratus* Kieffer, 1921, *Dicrotendipes pallidicornis* (Goetghebuer, 1934), *Polypedilum* sp., *Polypedilum (Tripodura) bicrenatum* Kieffer, 1921, *Polypedilum (Pentapedilum) sordens* (van der Wulp, 1875), *Micropsectra radialis* Goetghebuer, 1939, and *Tanytarsus* sp.

*Key words*: Chironomidae, new faunistic records, Hazar Lake, Türkiye.

Aydın, G.B. (2022). Contributions to the faunistic knowledge of Chironomidae (Diptera) of Turkey based on the adult males collected around Hazar Lake (Elazığ). *Journal of the Entomological Research Society*, 24(3), 375-387.
*Received: May 26, 2022* *Accepted: September 14, 2022*

## INTRODUCTION

Chironomidae are an essential part of aquatic and terrestrial ecosystems where adults and immatures constitute a significant food source for invertebrates and vertebrates (Epler, 2001; Aydın & Güher, 2017). The high abundance and diversity of immature stages and their ubiquitous nature make them important bioindicators of lentic and lotic habitats (Epler, 2001; Failla, Vasquez, Hudson, Fujimoto, & Ram, 2016; Özkan et al, 2010). These ecological roles of chironomids make sampling and species-level identification an essential and helpful biological tool for biomonitoring the trophy level of a lake (Failla et al, 2016). The identification of chironomid larvae to species level can be difficult for researchers (Oliver, 1971; Failla et al, 2016). Because adults hold more developed and specific identifying features, species-level definitions are more possible to be made (Failla et al, 2016). Due to the problems with species identification based on larvae, it is recommended to evaluate the larval and adult individuals together (Namayandeh & Beresford, 2012).

In the present study, we investigated adult chironomids collected around Hazar Lake. The Hazar Lake is one of the largest and deepest lake in Eastern Anatolia of Türkiye and hosts rich biodiversity, the potential for fisheries, and bird habitats. In addition, the lake has been designated as a Wetland of International Importance under the Ramsar Convention, and some of the beaches around the lake have blue flag by the European Environmental Education Foundation (Varol, 2019). The previous studies on chironomid fauna of the lake were only on larvae (Şahin & Baysal, 1972; Tellioğlu, Çitil, & Şahin, 2008; Bakır, 2012; Sarı, 2012). Therefore, to overcome deficiencies in identifying chironomids in this important freshwater, we collected and identified adults for the first time in this study.

## MATERIALS AND METHODS

The Hazar Lake is located between latitude 38° 31' N and longitude 39° 25' E (Fig. 1). It is a lake of tectonic origin and is at an altitude of 1238 m. We performed the sampling between July and August 2021 from 11 different stations around Hazar Lake (Fig. 1). The stations' names, sampling dates, and coordinates are shown in Table 1. We obtained the adult non-biting midge specimens through the reeds and grass near the lake using a sweeping net and then sprayed 70% ethanol on the collected specimens to prevent them from flying. We collected all adult chironomids kept in the sweeping net after alcohol spray with the help of a fine-lead clamp and preserved them in bottles containing 70% ethanol. We labeled all materials and transferred to the laboratory for identification. We made temporary slides in glycerine for preliminary data (subfamily, antenna structure, body coloration, thorax structure) and then done permanent preparation according to Wiederholm, 1989. We utilized Wiederholm (1989), Armitage, Cranston, & Pinder (1995), Langton & Pinder (2007), Saether & Oyewo (2008), Andersen & Mendes (2010), and Epler (2018) for the identification. We stored the materials at the Biology Department of Trakya University, Hydrobiology Laboratory, Edirne, Türkiye.

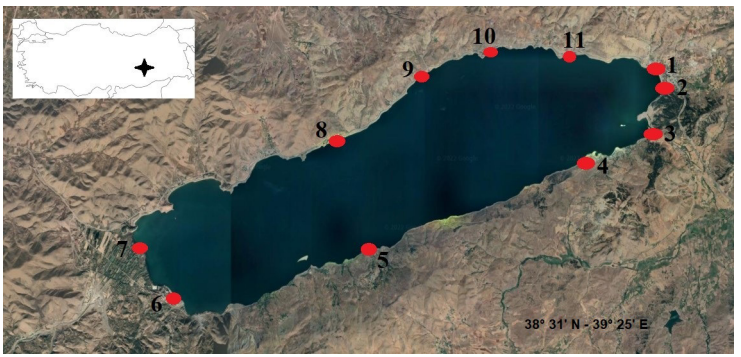


Fig. 1. The sampling stations around Hazar Lake (Elazığ), Türkiye (the numbers in the figure show the stations number in Table 1)

Table 1. Name, dates, and coordinates of the study sampling stations.

| NO | Stations | Sampling Dates | Coordinates |
|---|---|---|---|
| 1 | The Highway Maintenance | 19.07.2021, 24.07.2021, 02.08.2021, 05.08.2021, 07.08.2021 | 38°31'14"N, 39°30'33"E |
| 2 | Zıkkım Creek | 19.07.2021, 24.07.2021, 02.08.2021, 05.08.2021, 07.08.2021 | 38°30'55"N, 39°30'45"E |
| 3 | Behrimaz Creek | 24.07.2021, 02.08.2021, 05.08.2021, 07.08.2021 | 38°30'07"N, 39°30'24"E |
| 4 | Plajköy Camping | 20.07.2021, 10.08.2021, 15.08.2021 | 38°29'36"N, 39°29'07"E |
| 5 | The Old Railroads Camping | 20.07.2021, 10.08.2021, 15.08.2021 | 38°27'50"N, 39°23'54"E |
| 6 | Sivrice | 20.07.2021, 10.08.2021, 15.08.2021 | 38°26'59"N, 39°18'44"E |
| 7 | Kürk Creek | 20.07.2021, 10.08.2021, 15.08.2021, 18.08.2021 | 38°28'04"N, 39°17'43"E |
| 8 | The State Hydraulic Works Camping | 22.07.2021, 10.08.2021, 15.08.2021, 18.08.2021 | 38°29'46"N, 39°22'26"E |
| 9 | Fırat University Camping | 20.07.2021, 22.07.2021, 10.08.2021, 15.08.2021, 18.08.2021 | 38°01'06"N, 39°24'51"E |
| 10 | The 8. Region of Highways Camping | 20.07.2021, 22.07.2021, 24.07.2021, 02.08.2021, 05.08.2021, 07.08.2021 | 38°31'32"N, 39°26'29"E |
| 11 | Sevsak Creek | 19.07.2021, 22.07.2021, 24.07.2021, 02.08.2021 | 38°31'32"N, 39°28'38"E |

## RESULTS

We identified a total of 1103 individuals belonging to 17 species (subfamilies Tanypodinae, Orthocladiinae, and Chironominae) in this study (Table 2). We determined *Procladius (Holotanypus) choreus* (Meigen, 1804), *Cricotopus (Cricotopus) bicinctus* (Meigen, 1818), *Cricotopus (Cricotopus) festivellus* (Kieffer, 1906), *Cryptotendipes pseudotener* (Goetghebuer, 1922), *Cryptochironomus (Cryptochironomus) rostratus* Kieffer, 1921, *Dicrotendipes pallidicornis* (Goetghebuer, 1934), *Polypedilum* sp., *Polypedilum (Tripodura) bicrenatum* Kieffer, 1921, *Polypedilum (Pentapedilum) sordens* (van der Wulp, 1875), *Micropsectra radialis* Goetghebuer, 1939 ), and *Tanytarsus* sp.

for the first time around Hazar Lake. Also, we determined *Cryptotendipes pseudotener* (Goetghebuer, 1922) and *Dicrotendipes pallidicornis* (Goetghebuer, 1934) as new records for Turkish fauna.

Table 2. The Chironomidae species identified from Hazar Lake with their sampled station numbers (● = First record for the Hazar Lake, ♦ = First record for the Türkiye).

| Taxa | Station Number |
|---|---|
| **Tanypodinae** | |
| *Procladius (Holotanypus) choreus* (Meigen, 1804) ● | 1, 2, 3, 6, 7, 11 |
| *Tanypus (Tanypus) punctipennis* Meigen, 1818 | 1, 7, 8, 9, 10 |
| **Orthocladiinae** | |
| *Cricotopus (Cricotopus) bicinctus* (Meigen, 1818) ● | 2, 3, 7, 11 |
| *Cricotopus (Cricotopus) festivellus* (Kieffer, 1906) ● | 1, 11 |
| *Cricotopus (Isocladius) sylvestris* (Fabricius, 1794) | 2, 3, 11 |
| **Chironominae** | |
| *Chironomus (Chironomus) plumosus* (Linnaeus, 1758) | 1, 6 |
| *Cryptotendipes pseudotener* (Goetghebuer, 1922) ♦ | 1, 10 |
| *Cryptochironomus (Cryptochironomus) rostratus* Kieffer, 1921● | 1, 4, 5, 10 |
| *Dicrotendipes pallidicornis* (Goetghebuer, 1934) ♦ | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,11 |
| *Kiefferulus (Kiefferulus) tendipediformis* (Goetghebuer, 1921) | 1 |
| *Polypedilum* sp. ● | 1 |
| *Polypedilum (Tripodura) bicrenatum* Kieffer, 1921 ● | 1, 4, 5, 8, 9 |
| *Polypedilum (Polypedilum) nubifer* (Skuse, 1889) | 3 |
| *Polypedilum (Pentapedilum) sordens* (van der Wulp, 1875) ● | 4, 5 |
| *Cladotanytarsus (Cladotanytarsus) mancus* (Walker, 1856) | 1, 4, 6, 7, 8, 9, 10 |
| *Micropsectra radialis* Goetghebuer, 1939 ● | 1, 2 |
| *Tanytarsus* sp. ● | 1, 6 |

## Family Chironomidae

## Subfamily Tanypodinae

### *Procladius (Holotanypus) choreus* (Meigen, 1804)

Material examined: Stations 1, 2, 3, 6, 7, and 11; 233 ♂♂.

Distribution: Uluabat Lake (Arslan, Ayık, & Şahin, 2010).

### *Tanypus (Tanypus) punctipennis* Meigen, 1818

Material examined: Stations 1, 7, 8, 9, and 10; 37 ♂♂.

Distribution: Aras, Ceyhan, Fırat rivers and Van Lake (Şahin, 1984), Eğrigöl Lake (Yıldız, Taşdemir, Özbek, Balık, & Ustaoğlu, 2005), Akşehir, Çavuşcu, Gölhisar, Karataş lakes (Taşdemir & Ustaoğlu, 2005), Büyükçekmece Lake (Koşal-Şahin, 2006), Meriç River (Özkan & Çamur-Elipek, 2006), Meriç-Ergene River Basin (Özkan and Çamur-Elipek, 2006; Aydın & Güher, 2017; Aydın & Çamur-Elipek, 2019), Musaözü Dam Lake (Arslan et al., 2007), Büyük Stream (Kara, 2008), Hazar Lake (Tellioğlu et al, 2008), Uluabat Lake (Arslan et al, 2010), Ergene River (Özkan, Moubayed, & Çamur-Elipek, 2010), Gala Lake (Çamur-Elipek et al, 2010), Delice Stream (Rüzgar, 2010), Süleymanlı Lake (Duran & Akyıldız, 2011), Erikli and Hamam lakes (Çamur-Elipek, Güher, Kırgız, & Özkan, 2012), Sapanca Lake (Bakır, 2012), Paşalimanı Island (Özkan, 2012), Karamenderes, Kocabaş, Sarıçay, Tuzla streams (Odabaşı, 2013), Gönen Stream (Taşdemir, Aydemir-Çil, & Ustaoğlu, 2018), Abant Lake (Tüzün-Tereshenko, 2019), Dicle River (Çamur-Elipek et al, 2021).

**Subfamily Orthocladiinae**

***Cricotopus (Cricotopus) bicinctus* (Meigen, 1818)**

Material examined: Stations 2, 3, 7, and 11; 20 ♂♂.

Distribution: Aras, Asi, Ceyhan, Fırat, Kura rivers and Van Lake (Şahin, 1984), Gümüldür Stream (Ustaoğlu, Balık, and Taşdemir, 2005), Meriç River (Özkan & Çamur-Elipek, 2006), Meriç-Ergene River Basin (Özkan & Çamur-Elipek; Aydın & Güher, 2017), Tunca River (Çamur-Elipek, Arslan, Kırgız, & Öterler, 2006), Sazlıdere Stream (Özkan & Çamur-Elipek, 2007), Delice Stream (Rüzgar, 2010), Ergene River (Özkan et al, 2010), Gala Lake (Çamur-Elipek et al, 2010), Beyşehir and Eğirdir lakes (Bakır, 2012), Erikli, Hamam, Mert lakes (Çamur-Elipek et al, 2012), Karamenderes, Kocabaş, Sarıçay, Tuzla streams (Odabaşı, 2013), Dicle River (Çetinkaya & Bekleyen, 2017), Kızılırmak and Yeşilırmak estuaries (Rüzgar, 2019), Dicle River (Çamur-Elipek, Şen, Aydın, Taş-Divrik, and Yıldırm, 2021), Büyük Menderes River Basin (Akyıldız & Duran, 2021), Kanak Dam Lake (Taş-Divrik, Öz-Laçin, Kalkan, & Yurtoğlu, 2021), Dilderesi Stream (Bayköse et al, 2022).

***Cricotopus (Cricotopus) festivellus* (Kieffer, 1906)**

Material examined: Stations 1 and 11; 16 ♂♂.

Distribution: Fırtına Stream (Abed-Abed, 2021).

***Cricotopus (Isocladius) sylvestris* (Fabricius, 1794)**

Material examined: Stations 2, 3, and 11; 13 ♂♂.

Distribution: Meriç River (Özkan & Çamur-Elipek, 2006), Meriç-Ergene River Basin (Özkan & Çamur-Elipek; Aydın & Güher, 2017), Sazlıdere Stream (Özkan & Çamur-Elipek, 2007), Ergene River (Özkan et al, 2010), Gala Lake (Çamur-Elipek et al, 2010), Delice Stream (Rüzgar, 2010), Yuvarlakçay Stream (Taşdemir, Ustaoğlu, & Balık, 2010), Uluabat Lake (Arslan et al, 2010), Kapıdağ Peninsula (Özkan, 2011), Aygır,

Beyşehir, Çıldır, Eğirdir, Erçek, Hazar, Marmara, Sıhke lakes (Bakır, 2012), Erikli, Mert lakes (Çamur-Elipek et al, 2012), Saklıgöl Lake (Taşdemir & Ustaoğlu, 2016), Yeniçağa Lake (Sönmez, 2017), Abant Lake (Tüzün-Tereshenko, 2019), Kızılırmak and Yeşilırmak estuaries (Rüzgar, 2019), Büyük Menderes River Basin (Akyıldız & Duran, 2021), Sera Lake (Abed-Abed, 2021), Dilderesi, Yalakdere streams (Bayköse et al, 2022).

**Subfamily Chironominae**

***Chironomus (Chironomus) plumosus* (Linnaeus, 1758)**

Material examined: Stations 1 and 6; 16 ♂♂.

Distribution: Eğrigöl Lake (Yıldız et al, 2005), Akşehir, Beyşehir, Çavuşcu, Eber, Eğirdir, Gölhisar, Karamık, Karataş lakes (Taşdemir & Ustaoğlu, 2005), Büyükçekmece Lake (Koşal-Şahin, 2006), Meriç River (Özkan & Çamur-Elipek, 2006), Meriç-Ergene River Basin (Özkan & Çamur-Elipek; Aydın & Güher, 2017; Aydın & Çamur-Elipek, 2019), Tunca River (Çamur-Elipek et al, 2006), Hazar Lake (Tellioğlu et al, 2008), Büyük Stream (Kara, 2008), Kesikköprü Dam Lake (Ahıska, 2009), Delice Stream (Rüzgar, 2010), Ergene River (Özkan et al, 2010), Gala Lake (Çamur-Elipek et al, 2010), Tahtalı Dam Lake (Taşdemir, Yıldız, Özbek, Ustaoğlu, & Balık, 2010), Yuvarlakçay Stream (Taşdemir et al, 2010), Uluabat Lake (Arslan et al, 2010), Kapıdağ Peninsula (Özkan, 2011), Süleymanlı Lake (Duran and Akyıldız, 2011), Erikli, Hamam, Mert lakes (Çamur-Elipek et al, 2012), Paşalimanı Island (Özkan, 2012), Sapanca Lake (Bakır, 2012), Karamenderes, Kocabaş, Sarıçay, streams (Odabaşı, 2013), Gökçeova, Karagöl, Kartal lakes (Taşdemir & Ustaoğlu, 2016), Yeniçağa Lake (Sönmez, 2017), Abant Lake (Tüzün-Tereshenko, 2019), Yeşilırmak estuaries (Rüzgar, 2019), Uzungöl Lake (Abed-Abed, 2021), Dicle, Fırat rivers (Çamur-Elipek et al, 2021), Kanak Dam Lake (Taş-Divrik et al, 2021).

***Cryptotendipes pseudotener* (Goetghebuer, 1922) (Fig. 2A)**

Material examined: Stations 1 and 10; 21 ♂♂.

Distribution: We could not find any distributional records of this secies in Türkiye. In Europe the species is reported from Hungary (Mora, 2004), Finland (Paasivirta, 2014), Ireland (Murray, O'Connor, & Ashe, 2018), and Italy (Rossaro et al, 2019).

Diagnosis: Wing membrane bare, squama at the base of wing is fringed with seta. Eye bare and frontal tubercles absent. Palp well-developed. Antepronotum notched dorsally. Scutum is not overreaching antepronotum. Apex of fore tibia bears low and rounded projection. Combs of mid and hind tibiae narrowly separated and each bearing a spur. Pulvilli large and distinct. Inferior volsella absent. Anal tergite without processes next to the anal point. Anal point long, extending past mid-point of gonostylus. Superior volsella rod-like. Gonostylus long and incurved but not much swollen basally. Gonostylus with no apical tooth (Fig. 2A).

***Cryptochironomus (Cryptochironomus) rostratus* Kieffer, 1921**

Material examined: Stations 1, 4, 5, and 10; 56 ♂♂.

Distribution: Ereğli- Konya (Reiss, 1985).

***Dicrotendipes pallidicornis* (Goetghebuer, 1934) (Fig. 2B)**

Material examined: Stations 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11; 336 ♂♂.

Distribution: We could not find any distributional records of this species in Türkiye. In Europe the species is reported from France (Moubayed-Breil, 2007), Crete, Corsica, Great Britain, Ireland, the Netherlands, and Spain (Murray et al, 2018).

Diagnosis: Wing membrane bare, squama at the base of wing with long seta. Wing membrane with dark markings. Eye bare. Antepronotal lobes narrow and weakly notched medially. Scutum is not overreaching antepronotum. Fore tibia rounded scale and lacking spur. Combs of mid and hind tibiae composed of basally fused spinules and each with a long spur. Pulvilli simple, lobe-like, and as long as claw. Inferior volsella of the hypopygium distinctly forked at the tip (Fig. 2B).

***Kiefferulus (Kiefferulus) tendipediformis* (Goetghebuer, 1921)**

Material examined: Station 1; 2 ♂♂.

Distribution: Meriç-Ergene River Basin (Özkan and Çamur-Elipek; Aydın & Güher, 2017; Aydın & Çamur-Elipek, 2019), Kayalı Stream (Özkan, 2007), Hazar Lake (Bakır, 2012; Sarı, 2012), Marmara Lake (Bakır, 2012).

***Polypedilum* sp.**

Material examined: Station 1; 9 ♂♂.

***Polypedilum (Tripodura) bicrenatum* Kieffer, 1921**

Material examined: Stations 1, 4, 5, 8, and 9; 69 ♂♂.

Distribution: Ceyhan, Çoruh, Fırat, Kura rivers and Van Lake (Şahin, 1984), Meriç River (Özkan & Çamur-Elipek, 2006), Meriç-Ergene River Basin (Özkan & Çamur-Elipek; Aydın & Güher, 2017), Tunca River (Çamur-Elipek et al, 2006), Kayalı, Kiremitlik, Masıt, Münipbey, Pınarbaşı streams and Tayfur Dam Lake (Özkan, 2007), Kapıdağ Peninsula (Özkan, 2011).

***Polypedilum (Polypedilum) nubifer* (Skuse, 1889)**

Material examined: Station 3; 2 ♂♂.

Distribution: Dicle and Fırat rivers (Şahin, 1984), Beyşehir and Eğirdir lakes (Taşdemir & Ustaoğlu, 2005), Eğrigöl Lake (Yıldız et al, 2005), Gümüldür Stream (Ustaoğlu et al, 2005), Büyükçekmece Lake (Koşal-Şahin, 2006), Kovada Lake (Arslan & Şahin, 2006), Meriç River (Özkan & Çamur-Elipek, 2006), Meriç-Ergene River Basin (Özkan & Çamur-Elipek; Aydın & Güher, 2017; Aydın & Çamur-Elipek, 2019), Tunca River (Çamur-Elipek et al, 2006), Kocabaş Stream (Özkan, 2007), Büyük Stream (Kara, 2008), Delice Stream (Rüzgar, 2010), Ergene River (Özkan et al, 2010),

Kapıdağ Peninsula (Özkan, 2011), Gala, Çemek, Hazar, Marmara lakes (Bakır, 2012), Paşalimanı Island (Özkan, 2012), Karamenderes, Kocabaş, Tuzla streams (Odabaşı, 2013), Kufi Stream (Özcan, 2013), Kartal Lake (Taşdemir & Ustaoğlu, 2016), Abant Lake (Tüzün-Tereshenko, 2019).

***Polypedilum (Pentapedilum) sordens* (van der Wulp, 1875)**

Material examined: Stations 4 and 5; 11 ♂♂.

Distribution: Kovada Lake (Arslan & Şahin, 2006), Meriç-Ergene River Basin (Özkan & Çamur-Elipek), Gala Lake (Çamur-Elipek et al, 2010), Beyşehir Lake (Bakır, 2012; Sarı, 2012), Gölbaşı Lake (Arslan, Kara, & Odabaşı, 2013), Yeniçağa Lake (Sönmez, 2017), Yeşilırmak estuaries (Rüzgar, 2019).

***Cladotanytarsus (Cladotanytarsus) mancus* (Walker, 1856)**

Material examined: Stations 1, 6, 7, 8, 9, and 10; 169 ♂♂.

Distribution: Aras, Asi, Ceyhan, Çoruh rivers and Van Lake (Şahin, 1984), Beyşehir (Taşdemir & Ustaoğlu, 2005), Büyükçekmece Lake (Koşal-Şahin, 2006), Meriç River (Özkan & Çamur-Elipek, 2006), Meriç-Ergene River Basin (Özkan & Çamur-Elipek; Aydın & Güher, 2017; Aydın & Çamur-Elipek, 2019), Tunca River (Çamur-Elipek et al, 2006), Musaözü Dam Lake (Arslan et al., 2007), Münipbey, Pınarbaşı streams and Tayfur Dam Lake (Özkan, 2007), Sazlıdere Stream (Özkan & Çamur-Elipek, 2007), Büyük Stream (Kara, 2008), Hazar Lake (Tellioğlu et al, 2008), Delice Stream (Rüzgar, 2010), Ergene River (Özkan et al, 2010), Gala Lake (Çamur-Elipek et al, 2010), Kapıdağ Peninsula (Özkan, 2011), Beyşehir, Çıldır, Hazar, Sapanca lakes (Bakır, 2012), Sihke Lake (Sarı, 2012), Gölbaşı Lake (Arslan et al, 2013), Karamenderes, Kocabaş, Tuzla streams (Odabaşı, 2013), Kufi Stream (Özcan, 2013), Gökçeova Lake (Taşdemir & Ustaoğlu, 2016), Yeniçağa Lake (Sönmez, 2017), Abant Lake (Tüzün-Tereshenko, 2019), Yeşilırmak estuaries (Rüzgar, 2019), Dicle and Fırat rivers (Çamur-Elipek et al, 2021), Abdal, Aksu, Çataklıhoca, Fol, Hemşin, İkizdere, Karadere, Solaklı streams (Abed-Abed, 2021), Dilderesi, Kirazdere, Yalakdere (Bayköse et al, 2022).

***Micropsectra radialis* Goetghebuer, 1939**

Material examined: Stations 1 and 2; 82 ♂♂.

Distribution: Banaz Stream (Özcan, 2013), Kanak Dam Lake (Taş-Divrik et al, 2021),

***Tanytarsus* sp.**

Material examined: Stations 1 and 6; 11 ♂♂

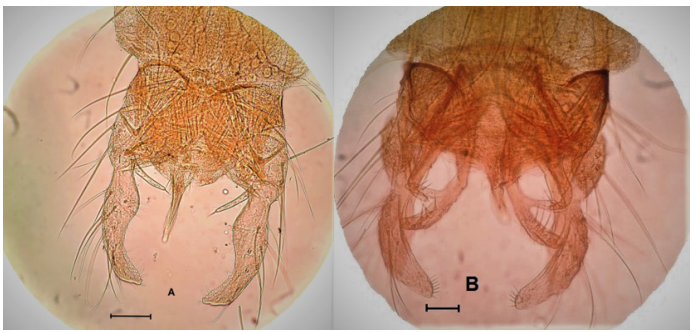


Fig. 2. The hypopygia of A: *Cryptotendipes pseudotener*, B: *Dicrotendipes pallidicornis* (the scale lines represent a distance of 40 µm).

## CONCLUSIONS AND DISCUSSION

In this study, adult male chironomids from the Hazar Lake in Elazığ, Türkiye were investigated. Hazar Lake is an internationally important freshwater within the scope of the Ramsar Convention (1971). The previous studies conducted in this lake have only been based on the identification of larval stage with a total of 24 chironomid species being identified so far (Şahin & Baysal, 1972; Tellioğlu et al, 2008; Bakır, 2012; Sarı, 2012). As a result of this investigation, we recorded a total of 11 species from three subfamilies of Chironomidae for the first time from the Hazar Lake increasing the number to 35. Chironominae was found to be the richest and most abundant subfamily in this freshwater with 12 species and 71% of all specimens with a total of 784 individuals (Table 2). This was followed by the subfamily Tanypodinae (25% with 270 individuals). The subfamily Orthocladiinae was found to be represented with the lowest individual numbers (4% with 49 individuals) (Fig. 3).

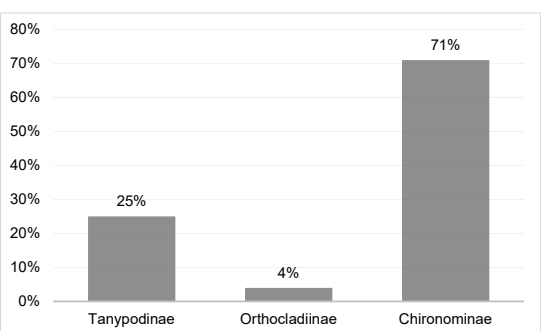


Fig. 3. The percentage of subfamilies by number of individuals.

The newly recorded *D. pallidicornis* had the highest abundance, with 336 individuals, among the species collected. The larvae of *D. pallidicornis* are tolerant of saline conditions (Murray et al, 2018). Previous studies reported saline conditions in the lake (Ünlü, Çoban, & Tunç, 2007; Rashid, 2022).

Limnologists generally use chironomid larvae to evaluate and monitor environmental conditions, the levels of organic pollution in streams, rivers, and wetlands (Kang, Baek, Kang, & Bae, 2022). However, chironomid larvae are usually difficult to identify to species level because compared to the immatures, the adult chironomids possess more developed and distinguishing features for species-level identification (Kang et al, 2022). Therefore, to build on the previous studies conducted on larval stages we obtain the data on the faunistic knowledge of adult Chironomidae; further facilitating the future studies on the ecology, distribution, and life history of chironomids of the region.

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*J. Entomol. Res. Soc., 24(3): 389-393, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2326* *Online ISSN:2651-3579*

# A New Species of the *Hilara intermedia*-group (Diptera: Empididae) from Turkey

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## ABSTRACT

*Hilara caglari* Çiftçi sp. nov. from *Hilara intermedia*-group of species is described from Sivas province (central Anatolia) of Turkey. The male genitalia and foreleg of the new species are illustrated and distinguished from other Palaearctic congeners. The *Hilara intermedia*-group from Europe and Middle East now includes a total of 27 species, 2 of which are distributed in Turkey.

*Key words: Hilara*, distribution, dance flies, new species, Sivas.

Çiftçi, M.C., (2022). A new species of the *Hilara intermedia*-group (Diptera: Empididae) from Turkey. *Journal of the Entomological Research Society,* 24(3), 389-393.

Received: August 24, 2022 Accepted: November 3, 2022

## INTRODUCTION

The *Hilara intermedia*-group was defined by Collin (1961) under the name *Hilara quadrivittata*-group with 11 species. Later, Chvála (2002, 2005, 2008) and Chvála and Merz (2009) revised the European *H. intermedia*-group and added new species to the group, recognizing 23 species from Europe and the Middle East. With the works of Çiftçi et al. (2012), Kustov et al. (2013) and Kanavalová et al. (2018), this number has increased to 26. Chvála (2002) divided the *H. intermedia*-group into three well-defined species complexes, the *H. intermedia* complex, *H. quadrifasciata* complex and *H. brevivittata* complex.

The *Hilara intermedia*-group is little known from Turkey, represented by only one species so far, *Hilara balikesirensis* Çiftçi, Hasbenli & Koç, 2012, described from northwest Anatolia (Çiftçi et al., 2012).

In this paper, a new species is described from Sivas, in central Anatolia. The *Hilara intermedia*-group is now represented in Europe and the Middle East by 27 species.

## MATERIAL AND METHODS

This study is based on three male specimens collected from Sivas province (central Anatolia) of Turkey in 2021. For illustration, the male genitalia and fore leg were dissected and cleared in 10% KOH for 24 h at 30°C. All figures were drawn using a binocular microscope with an ocular grid. After drawing, genital parts and fore leg were stored in a small capsule with glycerol and the capsule pinned along with the specimen. In this study, the morphological nomenclature of Cumming and Wood (2017) was followed.

The specimens in this study were collected by A. Hasbenli, Ü. Çağlar and Ş.B. Can during the post-construction monitoring studies within the scope of the Trans Anatolian Natural Gas Pipeline Project (TANAP) and are housed in the Zoological Museum of Gazi University (ZMGU).

## RESULTS

### *Hilara caglari* Çiftçi sp. nov. (Figs. 1-5)

Diagnosis. Medium sized species of the *Hilara intermedia*-group with dense light grey dusting. Body about 3-3.5 mm long, occiput dull grey and halter yellow. Scutum with four distinct black stripes in dorsal view, acrostichal bristles two-serial on apical half, three to four serial on posterior half of scutum. Prothoracic collar with long pale bristle on each side. Legs black, fore tibia with long dorsal bristles. Abdomen lighter grey dusting with pale hairs.

Description. *Male*. Head dark grey, face and frons light grey dusting and frons wider than base of postpedicel, face narrower. Occiput uniformly with grey dusting. Upper postocular bristles black, hairs below neck, much finer and paler. Equally long

ocellar and frontal bristles at least as long as postpedicel with stylus. Antenna black. Antennal stylus thickened, slightly shorter than postpedicel. Palpus black, grey dusting with fine, brownish ventral hairs, preapical bristle long, black. Labrum shiny black, longer than half length of head.

Thorax light grey, scutum with slightly light brownish dusting in lateral view. Scutum with four distinct black stripes in dorsal view, scutum with brownish dusting and two outer stripes almost invisible in anterior view. In posterior view, two outer stripes much wider and nearly converge in middle with two inner stripes. Bristles and hairs on thorax mostly black, only anterior part of scutum and humeral area with short pale hairs including first two rows of acrostichal and dorsocentral bristles. Acrostichal and dorsocentral bristles short, hair-like, much shorter than antennal stylus. Acrostichal bristles two-serial on apical half, three to four serial on posterior half; dorsocentral bristles uniserial, becoming longer posteriorly, ending with two rather long prescutellar pairs. Large marginal bristles fine, as long as two thirds of postpedicel with stylus: one fine humeral, one fine intrahumerals, three notopleurals, one long, two to three short and fine supra-alars, one postalar and two pairs of scutellar bristles. Notopleural depression anteriorly with small black hairs. Supra-alar and post-alar bristles longer than humeral and intrahumeral bristles, nearly as long as scutellar bristles. Prothoracic collar with long pale bristle on each side, between them with row of pale hairs, proepisternum and sides of prosternum with very short, fine pale hairs.

Wings almost clear, slightly brownish. Veins blackish, anal vein distinct at base. Pterostigma brownish, slightly visible. Costal bristle long and black. Squama pale with whitish fringes; halter light yellow with slightly brownish stem.

Legs short and stout, with black and greyish dusting. Coxae light grey like pleura with pale hairs; mid and hind coxae laterally with one or two black bristle-like fine hairs. All femora and tibiae with pale and black hairs, pale hairs shorter and finer. Fore femur posteroventrally with fine long hairs at least as long as depth of fore femur. Mid femur anteroventrally with black bristle-like hairs, much longer and thicker on apical half. Hind femur dorsally with black hairs as long as depth of hind femur. Hairs on tibiae denser than femora and pale hairs reduced. Fore tibia (Fig. 1) dorsally with one row of very long bristles and posteroventrally with fairly long and fine bristle-like black hairs and shorter fine pale hairs. Mid tibia anteriorly and posteriorly with long, dense brownish hairs and black bristles, black bristles slightly longer than hairs. Hind tibia with short black hairs, slightly longer dorsal bristle-like hairs as long as depth of hind tibia. Tarsal segments with short black hairs. Fore basitarsus (Fig. 1) oval slightly longer than half length of fore tibia and slightly wider than tip of fore tibia.

Abdomen light grey dusting as thorax, abdominal hairs short, longer and yellowish on basal segments, black on posterior segments. Hind marginal bristles rather short but distinct and black, only pale on first and second terga. Black hind marginal bristles much longer than pale ones. Genitalia (Figs. 2-5) rather small and darker coloured. Tip of hypandrium simple (Fig. 3), epandrial lamella posteriorly with long bristly hairs. Apical projection of epandrial (Figs. 4-5) lamella shining black, spinose with blunt tip.

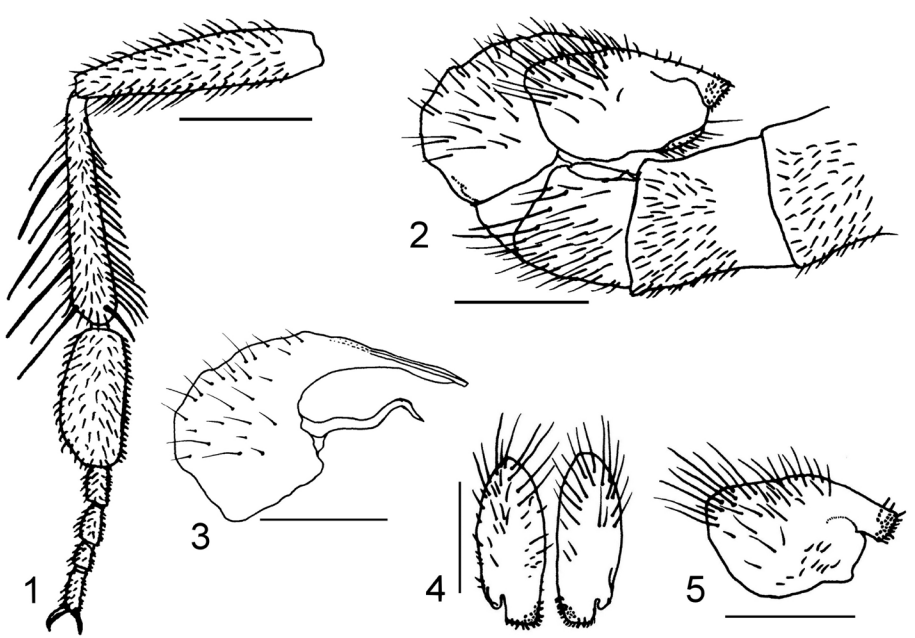
Length: Body 3-3.5 mm, wing 3.3-3.8 mm

Female. Unknown.

Type material. Holotype: Turkey, ♂, Sivas, Yıldızeli, Belcik village, 1260 m, 39°49'N / 36°15'E, 19.05.2021, det. M.C. Çiftçi. Paratypes: 2 ♂♂, same locality and date as holotype.

Derivatio nominis. This species is named after Dr. Üzeyir Çağlar who helped to collect the specimens.

Remarks. *Hilara caglari* Çiftçi sp. nov. is a species of *Hilara intermedia*-group by its densely grey dusting body with dull grey occiput, four dark-striped scutum and spinose apical projection of epandrial lamella and resembles *Hilara intermedia* (Fallén) and *Hilara tetragramma* Loew because of lighter grey dusting body, thoracic pattern and pale bristle on each side of prothoracic collar. *Hilara caglari* can be easily distinguished from *H. intermedia* by its spinose apical projection of epandrial lamella, where the apical projection of *H. intermedia* is small and needle-like. Also, *H. tetragramma* is distinguished from *H. caglari* by its larger body, the absence of prominent bristles on its legs and different shape of the epandrial lamella. *Hilara caglari* is more similar to *H. primula* Collin in being of medium-sized body, two-serial acrostichal bristles in front and epandrial lamella with blunt spinose apical projection. *Hilara caglari* is clearly differentiated from *H. primula* with characters such as lighter grey dusting body, pale bristle on each side of the prothoracic collar, shorter male fore basitarsus and fore tibia posteroventrally with long and fine bristle-like black hairs.




Figs. 1-5. *Hilara caglari* spec. nov. 1. Fore leg; 2. Postabdomen; 3. Hypandrium; 4. Epandrial lamella in dorsal view; 5. Epandrial lamella. Scale: 0,3 mm.

## ACKNOWLEDGEMENTS

The author wishes to thank the TANAP Natural Gas Transmission Inc. for their financial support, ENVY Energy and Environmental Investments Inc. for conducting post-construction monitoring and Prof. Dr. Abdullah Hasbenli (Ankara, Turkey), Assoc. Prof. Dr. Üzeyir Çağlar (Ankara, Turkey) and Dr. Şirin Bahar Can (Ankara, Turkey) for collecting specimens.

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*J. Entomol. Res. Soc., 24(3): 395-406, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2333* *Online ISSN:2651-3579*

# Preliminary Study of the Relationship Between Caddisfly Larvae and Environmental Variables in Şimşir Stream (Karabük, TURKEY)

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## ABSTRACT

Şimşir Stream is located in Yenice Forest, which has been selected as one of the 100 forest hotspots with high biodiversity and needs urgent protection, and the study area includes two protection zones. Determining the current situation in an area that has not yet been exposed to human influence helps to monitor the response of the rivers in cases that may arise later, such as climate change, pollution, land use, and morphological degradation. Trichoptera are one of the most important groups of macroinvertebrates used to assess such disturbances in streams. Trichoptera larvae were collected from 8 stations along the Şimşir Stream during 2019 and 2020 seasonally by kick-sampling. During this investigation, 17 genera belonging to 15 families were identified. Environmental variables were recorded to determine the relationship with larvae. Most of the stations, except the station located on the tributary, showed positive correlations with water velocity, dissolved oxygen, pH, electrical conductivity, and stream width. It was found that the rate of the boulder in the substrate was one of the most important variables affecting the distribution of larvae. The only station that was sampled in the tributary of the stream had different taxa from other stations, and this showed the contribution of the tributary to the fauna.

*Key words:* Yenice Forest, *Thremma anomalum*, *Helicopsyche bacescui*, forest hot spot, reference, condition.

Ekingen, P. (2022). Preliminary study of the relationship between caddisfly larvae and environmental variables in şimşir stream (Karabük, Turkey). *Journal of the Entomological Research Society*, 24(3), 395-406.

*Received: September 07, 2022* *Accepted: November 23, 2022*

## INTRODUCTION

Biological monitoring uses systematically the biological responses to determine environmental changes (Rosenberg & Resh, 1993). Benthic macroinvertebrates are commonly used as indicators in biomonitoring studies in running waters. This group is preferred because it consists of animals that tend to move less, have a wide species richness that can be affected to varying degrees by disturbances from different sources, and have a life cycle long enough to monitor (Reece & Richardson, 1999; Niemi & McDonald, 2004; Bae, Kil, & Bae, 2005). First of all, it is necessary to know the community structure and its relationship with undisturbed environmental variables in running waters where there is no human impact to carry out biological monitoring (Hodkinson & Jackson, 2005). This state, which is free from human influence, is known as the "reference condition". In the case of physical, chemical, or morphological degradation, the change in the habitat quality of the stream can be determined by understanding how much deviation is from this reference condition according to the presence, absence, or abundance data of macroinvertebrate (Council of European Communities, 2000). Trichoptera are one of the most important groups of macroinvertebrates used in biological monitoring studies to determine the effects of organic pollution (Dohet, 2002), climate change (Hering et al, 2009; Sáinz-Bariáin et al, 2016), metal pollution (Sola & Prat, 2006), morphological deterioration (Chakona, Phiri, & Day, 2009), and water velocity (de Brouwer, Besse-Lototskaya, ter Braak, Kraak, & Verdonschot, 2017) in aquatic ecosystems. They are found in almost all kinds of river habitats and the diversity of species on a wide scale for different river conditions has made the Trichoptera order a group that can be used in biological monitoring studies.

Yenice Forest was selected as one of the 100 forest hotspots with high biodiversity with urgently required protection by the World Wide Fund for Nature (WWF) in 1999, and it was determined as one of the Key Biodiversity Areas by the Doğa Derneği according to criteria set by the International Union for Conservation of Nature (IUCN) (Erciyas & Lise, 2006). It is shown as one of the 122 plant areas in Turkey that should be protected (Avcı, 2005). Yenice Forest, Turkey's largest uninterrupted forest (Erciyas & Lise, 2006), includes 3 different protection areas: Yenice Wildlife Development Area, Çitdere Nature Protection Area, and Kavaklı Nature Protection Area. The Şimşir Stream and Çitdere which are subjects of this project, are located in the first two protection areas.

Studies on benthic macroinvertebrates in the region are limited. Tanatmış (2004) conducted a study on the order Ephemeroptera found in the Filyos (Yenice) River and one of the 54 stations is located in the Şimşir Stream. Sipahiler (2014) collected adult Trichoptera specimens in Zonguldak and Karabük provinces, including Yenice Forest, and identified the species found in these regions. However, a study investigating the relationship of larvae with environmental variables has not been conducted in this region.

The goal of this paper was to determine the relationship between Trichoptera larvae and environmental variables in Şimşir Stream in Yenice Forest, which is currently far

from human influence. By comparing the future studies in this region with the data obtained from these studies, it can be determined whether there is any deterioration in the stream and the degree of deterioration.

## MATERIALS AND METHODS

### Study area

Şimşir Stream, which takes its source from Hodulca Hill in the Western Black Sea Region, passes through Şeker Canyon and mixes with the Filyos River, and discharges into the Black Sea (Fig. 1). The rugged topography restricted human activities in Yenice Forest, thus ensuring that the biodiversity of the region remains intact (Erciyas & Lise, 2006; Öztekinci & Coşkun, 2021).

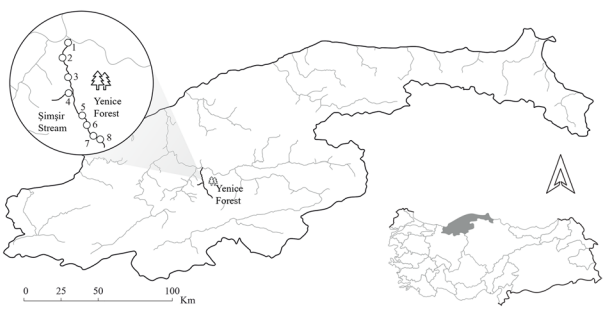


Fig. 1. Northwestern Anatolia and stations studied on Şimşir Stream.

Yenice Forest is Turkey's largest uninterrupted forest (Erciyas & Lise, 2006) and the most important reason for maintaining this integrity is its high average elevation and slope values; this slope rises up to 33% in Şimşir Stream (Öztekinci & Coşkun, 2021). In this forest, mixed natural old forests form the margins of the Şimşir Stream and Çitdere catchment areas (Erciyas & Lise, 2006). The basin where the Şimşir Stream is located has Cretaceous lithological units and contains limestone (Coşkun, 2020).

### Sampling and identification

Benthic macroinvertebrate larvae were collected seasonally from 8 stations along the stream during 2019 (summer, autumn) and 2020 (spring, summer, and autumn) by kick-sampling (D-frame net with 500 µm mesh size). While the 7 stations were located on the main branch of Şimşir Stream, only one station (the 4th station) was located on the tributary of the stream. Other tributaries of the stream were also selected for sampling, but this could not be done because they were dry most of the year. Collected samples were passed through sieves with different mesh sizes to separate unwanted materials such as stones, leaves, and twigs, and they were taken to the laboratory by adding ethyl alcohol. Trichoptera larvae were separated and identified with the Leica EZ4 stereomicroscope to the lowest possible systematic level. In cases where no distinction could be made at the genus level, the two genera were given together (such as Ser/Sch). Due to the high endemism rate of Trichoptera species in Turkey,

the fact that the diagnoses are mostly made on males, and the lack of association studies of larvae with adults, it is often not possible to diagnose larvae at the species level. Larvae can be described by methods such as metamorphotype, DNA barcoding, rearing of larvae, or raising eggs from adults, but these studies are very limited for this group in Turkey (Sipahiler, 2007, 2013a, 2013b; Ekingen & Kazancı, 2019).

**Environmental data**

Environmental variables such as pH, electrical conductivity, dissolved oxygen, and temperature were measured in situ using Hanna 98194, simultaneous with benthic invertebrate sampling. Water velocity was measured by Hydro-bios rod held current meter. Variables such as the riparian vegetation, the structure of the stream substrate, shading, and stream width were recorded visually. Cummins (1962) and Harrelson, Rawlins, and Potyondy (1994) were followed to determine the size of the substrate. The two categories given as “pebble” (16-64 mm) and “gravel” (2-26 mm) are combined and given as a single category (gravel) between 2-64 mm. The CANOCO program (Ter Braak &Smilauer, 2002) was used to determine the effects of these environmental variables on the distribution of Trichoptera larvae. Variables showing high correlation with each other were not included in the analysis.

## RESULTS AND DISCUSSION

The sampled stations are located between 188 m a.s.l and 1001 m a.s.l (Fig. 2). The dissolved oxygen values were measured between 7.6 and 13.91 mg/l, whereas the water temperature ranged from 10.41 to 19.53 °C. The alkalinity of pH values ranging from 7.58 to 8.65 is related to the geological structure of the region containing limestone (Coşkun, 2020). Electrical conductivity values were measured between 76 and 385 µS/cm. The lowest electrical conductivity values were measured at the 4th station, which is a tributary of the stream, during all periods. The stream width at the sampled stations varies between 2 and 12 m. It was observed that the stream water velocity varied between 0.20 and 1.02 m/s. Since vegetation and shading around the stream were intense and similar at all stations, these environmental variables were not included in the analysis.

Sipahiler (2014) stated that 29 adult species belonging to 26 genera and 18 families inhabited Karabük province. During the study, 17 genera belonging to 15 families were found (Table 1). *Rhyacophila* Pictet, 1834 and *Hydropsyche* Pictet, 1834 larvae were found in most of the samples (94.4% and 91.6%, respectively), and in this respect, it is similar to a study conducted in rivers in the Eastern Black Sea Region (Ekingen & Kazanci, 2021). In the CCA analysis, the first and second axes show strong species-environment correlations (r: 0.95 and r: 0.70, respectively) (Table 2). While the first axis in the CCA ordination diagram represents 48% of the variations that can be explained by environmental variables, the two axes together represent 66% (Monte Carlo perm. Test, p-value: 0.020).

Table1. List of genera/species found in the stations in Şimşir Stream during the study.

| Family | *Genus/Species* | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 |
|---|---|---|---|---|---|---|---|---|---|
| Rhyacophilidae | *Rhyacophila* | * | * | * | * | * | * | * | * |
| Glossosomatidae | *Glossosoma* | | * | * | * | * | | | |
| Philopotamidae | *Philopotamus* | | | | * | | | * | * |
| | *Wormaldia* | | | * | * | | | | |
| Polycentropodidae | *Plectrocnemia* | | | * | | | | | |
| Hydropsychidae | *Diplectrona* | | | | * | * | | | |
| | *Hydropsyche* | * | * | * | * | * | * | * | * |
| Psychomyiidae | *Tinodes* | * | | | | | | * | |
| Brachycentridae | *Micrasema* | | * | * | * | | * | * | * |
| Uenoidae | *Thremma anomalum* | | | | * | | | | |
| Goeridae | *Lithax/Silo* | | | * | * | * | | * | |
| Lepidostomatidae | *Lepidostoma/Dinarthrum* | | | | | * | | * | |
| Limnephilidae | *Chaetopteryx* | | | | | | | | * |
| Sericostomatidae | *Oecismus* | | | | | * | | | |
| | *Sericostama/Schizopelex* | | | * | * | * | * | * | * |
| Beraidae | *Beraeamyia* | * | | | * | * | | | * |
| Helicopsychidae | *Helicopsyche bacescui* | | | | * | | | | |

Table 2. Results of canonical correspondence analysis (CCA).

| Axes | 1 | 2 | 3 | 4 | Total inertia |
|---|---|---|---|---|---|
| Eigenvalues: | 0.333 | 0.133 | 0.098 | 0.05 | 2.031 |
| Species-environment correlations: | 0.947 | 0.703 | 0.621 | 0.646 | |
| Cumulative percentage variance | | | | | |
| of species data: | 17 | 23.8 | 28.9 | 31.4 | |
| of species-environment relation: | 47.3 | 66.2 | 80.2 | 87.2 | |
| Sum of all eigenvalues | | | | | 1.955 |
| Sum of all canonical eigenvalues | | | | | 0.704 |

According to the CCA analysis (Fig. 3), the first three variables that best explain the change in the distribution of the larvae were stream width, altitude, and the percentage of the boulder on the stream substrate. It was seen that the stations were mostly ordered according to the altitude. (Fig. 3). Most of the stations showed positive correlations with water velocity, dissolved oxygen, pH, electrical conductivity, and stream width.

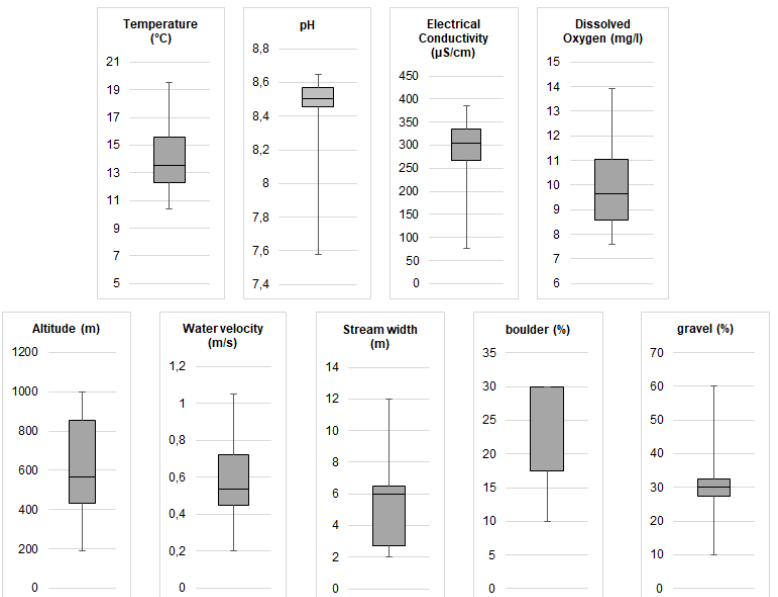


Fig. 2. Boxplot of nine environmental variables.

Station 4, unlike other stations, was not located on the main branch of Şimşir Stream, but it was sampled on a tributary at 567 m a.s.l. According to the CCA graph, Station 4 was located in a different place than the other stations (Fig. 3). The physicochemical variables that distinguished station 4 from most of the other stations were stream width, pH, electrical conductivity, and water velocity. This difference was also observed in the composition of Trichoptera in the station. In this study, *Thremma anomalum* McLachlan, 1876 and *Helicopsyche bacescui* Orghidan and Botosaneanu, 1953 were only found at this station. The habitats of the genera of the Uenoidae family, to which the genus *Thremma* belongs, are restricted to cold and small mountain streams (Vineyard & Wiggins, 1988). Although it was stated in the literature that *T. anomalum* was found only in cold waters near the headwaters (Graf, Murphy, Dahl, Zamora-Munoz, & Lopez-Rodriguez, 2008), Živić et al (2013) stated that the habitat of *T. anomalum* was not limited to cold waters only, so the classification as a cold stenotherm (<10°C) should be re-evaluated. In another study, it was observed that the water temperature varies between 5.2 and 16.8 °C in rivers where *T. anomalum* was found (Waringer, González, & Malicky, 2020). Likewise, *H. bacescui* is classified as a cold stenotherm (<10°C) in the literature (Graf et al, 2008), but the highest water temperature at the station (4th station) where these two species were found was 16.99°C in summer. According to the results, the presence of *T. anomalum* and *H. bacescui* are not limited to cold streams, but the reason why these species are not found in the main branch of the stream may be the high ratio of boulders and gravel in the substrate structure of the station where these species were found. In the literature (Vineyard & Wiggins, 1988; Živić, Markovic, Simic, & Kucinic, 2009; Živić et

al, 2013) it has been stated that the regions where these species live are rivers with boulder substrate. On the other hand, Station 4, has a high level of gravel besides its boulder substrate.

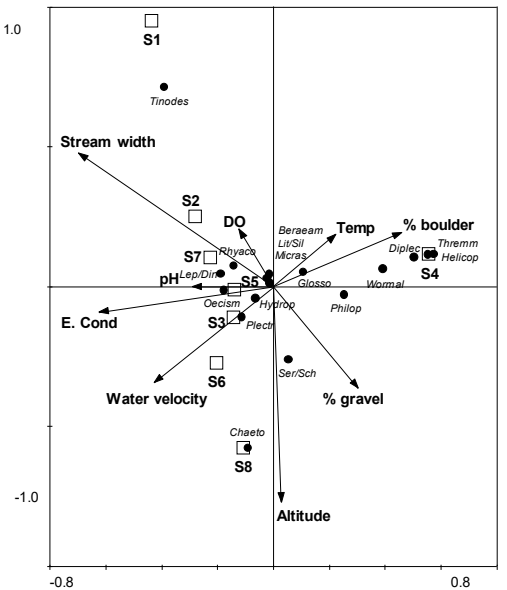


Fig. 3. CCA diagram showing the relationship between environmental variables and distribution of Trichoptera larvae. (Themma) *Thremma anomalum*, (Helicop) *Helicopsyche bacescui*, (Philop) *Philopotamus*, (Wormal) *Wormaldia*, (Diplec) *Diplectrona*, (Micras) *Micrasema*, (Lit/Sil) *Lithax*/*Silo*, (Beraeam) *Beraeamyia*, (Ser/Sch) *Sericostoma*/*Schizopelex*, (Glosso) Glossosoma, (Chaeto) *Chaetopteryx*, (Rhyaco) *Rhyacophila*, (Hydrop) *Hydropsyche*, (Plectr) *Plectrona*, (Oecism) Oecismus, (Lep/Din) *Lepidostoma*/*Dinarthrum*

A scale of the vulnerability of the species to climate change was created, and the species were matched with numbers between 0 and 6 on this scale: A value of 0 indicates that the effect is low, while a value of 6 indicates that the effect of climate change is high (Hershkovitz, Dahm, Lorenz, & Hering, 2015). *T. anomalum* and *H. bacescui* have a value of "2" in the literature (Graf et al, 2008). This means that they will not be affected much by climate change. However, in this study, these two species were found only at the station in the tributary of the stream (Station 4), and this station was one of the rare tributaries that did not dry out in all seasons. Although these species will not be affected or slightly affected by the increase in temperature that will occur with the effect of climate change, they may disappear locally due to the drying of the tributary of the stream. It has been reported that the drought caused by climate change will seriously affect the biota of the rivers, especially in the middle latitudes, and drought periods may be prolonged in temporary waters, and permanent waters may become temporary and even dry up completely (Fenoglio, Bo, Cucco, Mercalli, & Malacarne, 2010). Fenoglio, Bo, Cucco, & Malacarne (2007) observed that the abundance and taxonomic richness of macroinvertebrates in the hyporheic zone during dry periods in streams decreased as the drought period lengthened. Although

it is stated in the literature that *H. bacescui* is frequently found in periodically drying streams (Živić et al, 2009) these adaptive abilities of the species may not work if the natural regime of the stream is disturbed.

Information about the pH value of the stream in which *T. anomalum* inhabits was found in only one publication and it was stated that it ranged from 7 to 7.7 (Živić et al, 2013). In this study, the values measured at Station 4, the only station where *T. anomalum* was found, were measured between 7.58 and 8.53 throughout the study, and the pH values at this station during the periods when *T. anomalum* was present varied between 8.40 and 8.48. According to the CCA graph, the 4th station has a low pH value and although the species found here seem to prefer low pH values, it is because the lowest pH value measured at all stations during the study belongs to this station at 7.58.

Two genera from the Hydropsychidae family were found in this study: *Diplectrona* Westwood, 1840 and *Hydropsyche*. *Diplectrona* is similar to *T. anomalum* and *H. bacescui* in terms of river region, altitude, substrate structure, water velocity, temperature preferences (cold stenotherm), and sensitivity to climate change (Graf et al, 2008). Only *D. atra* of the genus *Diplectrona*, which is represented by five species in Turkey, is found in the Western Black Sea region, and its adult was previously recorded by Sipahiler (2019) in Yenice Forest.

*Hydropsyche* is a genus represented by 75 species in Turkey (Darılmaz & Salur, 2015; Sipahiler, 2016a). Longitudinal studies of this genus in rivers have reported that there are different Hydropsychids species with different feeding habits at different altitudes (Bing, Müller, Glaser, Brandl, & Brändle, 2015). In this study, *Hydropsyche* appears to show a wide ecological preference by placing close to the middle in the CCA graph. Since this genus could not be identified at the species level using larvae, the different ecological demands of the species belonging to this genus could not be determined and therefore it was located in the middle of the graph. The positive relationship with the water velocity may be related to the dominant feeding habits of this genus by passive filtering. Looking at the longitudinal distribution of hydropsychids in uncontaminated streams of England, it was found that they were lined up as *Diplectrona felix*- *Hydropsyche* spp.- *Cheumatopsyche lepida* (Edington & Hildrew, 1995). In this study conducted in the Western Black Sea region, although *Hydropsyche* was found at all stations, *Diplectrona* was found at stations close to the source. In another study conducted in the Eastern Black Sea Region (Ekingen & Kazanci, 2021), *Hydropsyche* was found in most stations, while larvae belonging to the *Cheumatopsyche* genus were found in the lower parts of the stream. In this respect, the longitudinal distribution of Hydropsychids at the species level which Edington & Hildrew (1995) stated, seems to be valid at the genus level in the Black Sea Region of Turkey as well.

The ecological preferences of most of the genera found in the study could not be interpreted due to the fact that they were located near the middle of the CCA graph. The reasons for being in the middle may be that they have a wide ecological tolerance, or that there are different species with different ecological requirements in the genus.

With this study, it has been observed that the larvae found here can also be found in alkaline waters in general.

## CONCLUSION

Yenice Forest is one of the least disturbed forest habitats in Turkey, but it is still known to be affected by forestry activities (Avcı, 2005; Öztekinci & Coşkun, 2021). The increase in road networks in the forest, the emphasis on the use of machinery in forest cutting, and illegal tree cutting are seen as threats that will affect Yenice Forest (Öztekinci & Coşkun, 2021). On the side of Şimşir Stream, there is a road opened for the transportation of forest products. It has been stated that no matter how small and sensitive forest activities are, they can cause physical deterioration that can harm aquatic ecosystems (Gökbulak, Serengil, Özhan, Özyuvacı, & Balcı, 2008) and that forestry activities will increase the influx of sediment into the stream and cause long-term damage to the invertebrates living here with the accumulation of sediment in the stream bed (Campbell & Doeg, 1989) but there is no study on effects of timber harvesting in this region.

The taxa, which are not found in other stations except for the tributary, show the contribution of the tributaries of the river to the fauna. Other tributaries were not sampled in this study as they were dry in some periods, but sampling them in the future will contribute to the determination of the fauna.

The fact that larval identifications cannot be made at the species level hinders the comparison and discussion of ecological information. The high level of endemism for Trichoptera in Turkish rivers makes the use of larval identification keys in other regions inconvenient for larval identification. For example, it is known that 50% of the *Tinodes* genus, which is represented by 32 species in Turkey, is endemic (Sipahiler, 2016b). The reason why some genera and variables could not be included in the discussion in this study is that ecological information at the species level is mostly not common at the genus level. Studies on the association of larvae and adults will not only enable the description of larvae but also increase the knowledge on the ecology of endemic species and make it possible to compare and use this information in future studies. Prioritizing the association studies between larvae and adults in later studies will be useful in determining the ecological preferences of the larvae and evaluating the river conditions.

Especially in biological monitoring studies, the determination of the changes in the rivers and taking measures accordingly requires the existence of reference data obtained from previous studies in the region. Since Yenice Forest has high biodiversity, is Turkey's largest uninterrupted forest, and is a region where human influence is low due to the topographic structure, the data to be obtained from the studies to be carried out in this region will be important in the evaluation of changing environmental conditions in the future.

## ACKNOWLEDGMENT

This study was supported by Hacettepe University Scientific Research Projects Coordination Unit (Project no. FHD-2019-18168, Determination of Trichoptera (Insecta) Fauna in Şimşir Stream (Yenice, Karabük) and Investigation of Its Relationship with Environmental Variables). The author would thank Prof. Dr. Muzaffer Dügel (Abant İzzet Baysal University) for his help on the data analysis of the study, Aykut Abdik and Dr. Çiğdem Özenirler (Hacettepe University) for their help in the field, and Ceren Şengül (Hacettepe University) for her help in the laboratory throughout the project.

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*J. Entomol. Res. Soc., 24(3): 407-415, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2322* *Online ISSN:2651-3579*

# *Carebara periyarensis* sp. nov. (Hymenoptera: Formicidae) a New Species from India

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## ABSTRACT

A new species of the genus *Carebara* Westwood, 1840, *Carebara periyarensis* sp. nov. is described and illustrated based on the worker caste. The new species resembles *C. affinis* and *C. diversa*, having 11 segmented antenna, however it can be differentiated from them by following combination of characteristics; small body size, short propodeal spine and body sculpture. An identification key to the genus *Carebara* is also updated.

*Key words:* Myrmicinae, new species, key, India, taxonomy.

Dhadwal, T., Bharti, H., (2022). *Carebara periyarensis* sp. nov. (Hymenoptera: Formicidae) a new species from India. *Journal of the Entomological Research Society,* 24(3), 407-415.

Received: August 18, 2022 Accepted: November 11, 2022

## INTRODUCTION

The genus *Carebara* Westwood, 1840 is one of the largest ant genera in the subfamily Myrmicinae. It is represented by 224 species and 22 valid subspecies worldwide. The genus is extensively dispersed throughout the tropical and subtropical regions of the world (Bolton, 2022). The majority of *Carebara* species are hypogaeic ants that build their nests in the ground and leaf litter. Whereas certain species appear to be having lestobiotic mode of living and some of which exhibit a severe size dimorphism between females and workers (Hölldobler & Wilson, 1990; Fernández, 2010).

Currently, on the basis of morphological and molecular analysis *Carebara* is considered as a senior synonym of *Afroxyidris*, *Oligomyrmex*, *Paedalgus*, *Parvimyrma* and *Pheidologeton* (Fernández, 2004, 2006, 2010; Moreau et al., 2006; Fischer et al., 2014; Moreau & Bell, 2013). Ward et al. (2015) assigned genus *Carebara* to the tribe Crematogastrinii. Formely it was treated in tribe Pheidolini (Emery, 1877), in Solenopsidini (Forel, 1893), in Myrmicariini (Ashmead, 1905) and in Pheidologetini (Emery, 1914; Bolton, 1994).

The significant contributions to the taxonomy of the genus from different parts of the world includes; from China (Li & Tang, 1986; Wu & Wang, 1995; Zhou & Zheng, 1997; Xu, 2003; Zhou et al., 2006), from Taiwan (Terayama et al., 2012), from Arabian Penninsula (Sharaf & Aldawood, 2013), from Afrotropical and Malagasy regions (Fischer et al., 2014, 2015; Azorsa & Fisher, 2018), from Thailand (Jaitrong et al., 2021), from Combodia (Fernández & Serna, 2019; Hosoishi et al., 2022).

Pertaining to the present study contribution from India includes Sheela & Narendran (1997), Bharti & Kumar (2013), Bharti & Akbar (2014), Akbar & Bharti (2017). A total of 25 species of genus *Carebara* are discovered so far (Bharti et al., 2016 ; Akbar & Bharti, 2017). However, there are still more ant species from India that need to be documented (Bharti et al., 2016). As a result, contributions in the form of new species and records are rather important. During the present study we describe a new species to the genus *Carebara* and also updated the identification key of the genus.

## MATERIALS AND METHODS

Taxonomic analysis was conducted on a Nikon SMZ 1500 stereo zoom microscope with maximum magnification of 112.5X. Digital images f of the specimens were prepared using a MP (Micro Publisher) digital camera and Auto Montage (syncroscopy, a division of Synoptics Ltd.) software. Images were cleaned with Adobe Photoshop CS5 and Helicon Filter 5. Morphological measurements were recorded in millimeters with an oculometer fitted on a Nikon SMZ 1500 stereomicroscope. Automontage images of specimens were provided by http://www.antweb.org/. Morphological terminology and standard measurements follow Hosoishi et al. (2022).

**HL (head length)**: maximum length of head in full-face view between lines drawn across anterior margin of clypeus and lines drawn across the posterolateral corners of head.

**HW (head width**): width of head directly behind the eyes measured in full-face view.

**SL (scape length)**: maximum scape length excluding basal condyle and neck.

**ML (mandible length)**: the straight-line length of the mandible at full closure from the mandibular apex to the clypeal margin.

**EL (eye length**): maximum diameter of compound eye measured in oblique profile view.

**EM (eye to mandible)**: distance from base of compound eye to the mandibular insertion, measured in profile view.

**WL (Weber's length**): diagonal length of mesosoma in profile view from the postero-ventral margin of the propodeal lobe to the anterior-most point of the pronotal slope, excluding the neck.

**PW (pronotal width)**: maximum width of pronotum measured in dorsal view.

**MFL (femur length)**: length of the profemur measured along its long axis in posterior view.

**MTL (tibia length**): maximum length of hind tibia.

**PTL (petiole length)**: maximum length of petiole, measured in profile view.

**PTH (petiolar node height)**: maximum height of petiolar node measured in profile view from the highest (median) point of the node to the ventral outline.

**PPL (postpetiole length)**: maximum length of postpetiole measured in profile view.

**PPH (postpetiole height**): maximum height of the postpetiole measured in profile view from the highest (median) point of the node to the ventral outline. The measuring line is placed at an orthogonal angle to the ventral outline of the node.

**PPW (postpetiole width**): maximum width of postpetiole measured in dorsal view.

**GL (gaster length)**: maximum length of the gaster measured in profile view.

**GW (gaster width)**: maximum width of the gaster measured in dorsal view.

**TL (total length):** measured roughly from the anterior margin of head to the tip of gaster in fully stretched specimens in profile.

**CI cephalic index**: 100*HW/HL.

**MI mandibular index**: 100*ML/HL.

**SI scape index**: 100*SL/HL.

**MLI metafemur length index**: 100*MFL/HL.

**PPLI postpetiole length index**: 100*PPL/PTL.

**PPI postpetiole width index:** 100*PPW/PTW.

**Depositories.**

PUAC "Punjabi University Patiala Ant Collection" at Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India.

## RESULTS

### *Carebara periyarensis* sp. nov. Figs (1-6)

Type Material: Holotype (worker): India: Kerala: Periyar Tiger Reserve, 930m, 9.3230°N, 77.1315°E, 27.i.2017 [PUAC T 101], Paratype; 6 (w.) with same data as holotype, Hand picking method, leg. Tarun Dhadwal [PUAC T 103-108 ].

#### Measurements

Holotype major worker: HL 1.03; HW 0.98; SL 0.57; ML 0.68; EM 0.36; WL 0.94; PW 0.58; MFL 0.66; MTL 0.51; PTL 0.30; PTH 0.22; PTW 0.16; PPL 0.28; PPH 0.20; PPW 0.26; GL 0.99; GW 0.70;CI 95.14; MI 66.01; SI 55.33; MLI 64.077; PPLI 93.33; PPI s162.50; TL 3.54 mm.

Paratype major workers (n=2): HL 1.01-1.06; HW 0.96-0.99; SL 0.52-0.57; ML 0.65-0.75; EM 0.33-0.39; WL 0.90-0.97; PW 0.54-0.63; MFL 0.64-0.69; MTL 0.51-0.53; PTL 0.30-0.33; PTH 0.22-0.24; PTW 0.14-0.16; PPL 0.24-0.28; PPH 0.21-0.22; PPW 0.25-0.28; GL 1.09-1.12; GW 0.74-0.84;CI 93.39-95.04; MI 64.35-70.75; SI 51.48-53.77; MLI 63.36-65.09; PPLI 80.00-84.84; PPI 175-178.57; TL 3.54-3.76 mm.

Paratype minor worker (n=4): HL 0.42-0.47; HW 0.45-0.49; SL 0.35-0.39; ML 0.28-0.31; EM 0.14-0.17; WL 0.52-0.55; PW 0.28-0.31; MFL 0.36-0.39; MTL 0.32-0.35; PTL 0.19-0.20; PTH 0.14-0.16; PPL 0.15-0.17; PPH 0.13-0.15; PTW 0.11-0.12; PPW 0.14-0.16; GL 0.48-0.53; GW 0.32-0.37; CI 104.25-107.14; MI 65.95-66.66; SI 82.97-83.33; MLI 82.97-85.71; PPLI 78.94-85.00; PPI 127.27-133.33; TL 1.76-1.92 mm.

#### Description of major worker (Figs.1-3)

Head in full face view as long as broad, posterior margin concave in the middle with occipital corners rounded, lateral sides convex anteriorly; median portion of clypeus bicarinate and divergent anteriorly, anterior margin weakly concave; mandibles with 5 teeth; antennae slender, 11 segmented with a two segmented club, scape short reaching up to half of the head length; eyes small with 3 pair of ommatidium.

In profile view, promesonotum high and convex, promesonotal suture obsolete, metanotal groove deeply impressed; propodeum with a pair of short denticles; propodeal declivity slightly concave; petiole node thick and subtriangular furnished with a keel beneath; postpetiole subglobose in shape. In dorsal view, pronotum globular, large, strongly convex occupying most of mesosoma; mesonotum small, transverse, metanotum reduced; petiole emarginated; postpetiole with convex lateral sides.

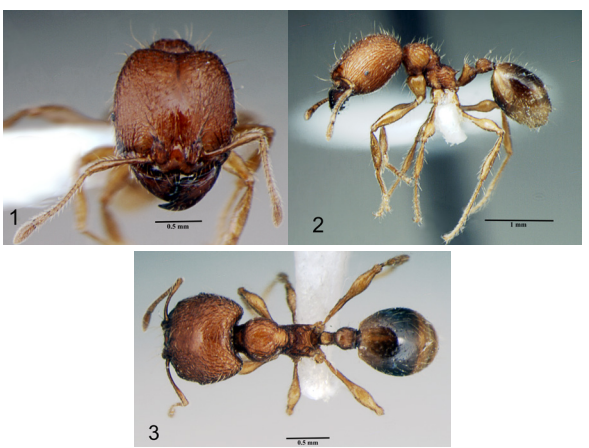
Mandibles longitudinally striated and clypeus smooth and shiny. Head finely and longitudinally striate throughout except small smooth median area at the frontal region, occiput with transverse striations; promesonotum longitudinally striated, metanotum reticulate and propodeum reticulate with few transverse striations; lateral sides of pronotum and propodeum with transverse striations; mesopleuron reticulated; in lateral view petiole and postpetiole with transverse striations and microreticulate in dorsal view, with one or two longitudinal rugae on petiole; postpetiole dorsally smooth and gaster reticulate anteriorly and rest of gaster smooth with punctures.

Head and body with abundant erect to suberect hairs; scapes and tibiae with dense decumbent pubescence.

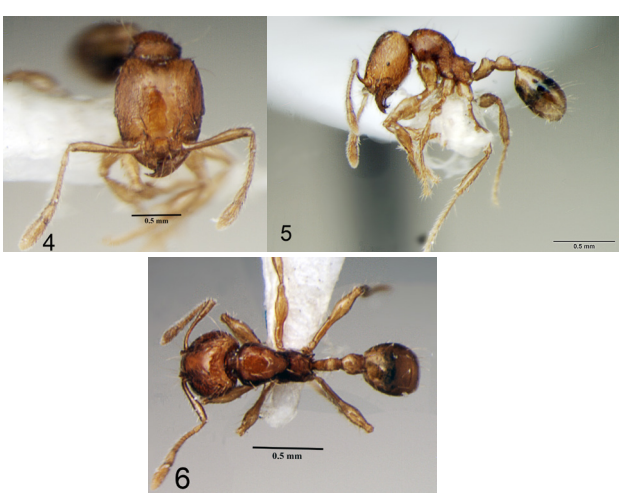
Body generally reddish yellow in color, head somewhat darker in color and legs lighter yellowish in color.

**Minor worker (Figs. 4-6)**

All characteristics are same except; size of minor worker is small, posterior margin widely emarginated in the middle, occipital corners almost straight, slightly rounded; mandibles smooth, head feebly longitudinally striate at sides, having smooth median portion and reticulated at occipital corners; pronotum smooth feebly reticulated anteriorly, mesonotum and metanotum reticulated, mesopleuron and metapleuron minutely reticulated. Body generally with less hair as compared to major worker.




Figs 1-3. Major worker of *Carebara periyarensis* sp. nov. 1. head in full face view 2. body in profile view 3. body in dorsal view.




Figs. 4-6. Minor worker of *Carebara periyarensis* sp. nov. 4. head in full face view, 5. body in profile view 6. body in dorsal view.

Queen. Unknown.

Male. Unknown.

Differential diagnosis. *C. periyarensis* sp. nov. can be differentiated from the already described species from India on the basis of antennal segment as new species have 11 segmented antenna while rest of the species have 9 segmented antenna excluding *Carebara affinis* (Jerdon, 1851) and *Carebara diversa* (Jerdon, 1851) having 11 segmented antenna.

Though, it can be differentiated from *C. affinis* on the basis of following characteristics (Fig. 7 A, B and Fig. 7 C, D); Head in *C. periyarensis* sp. nov. is as long as broad (head is distinctly longer than broad in *C. affinis*); in *C. periyarensis* sp. nov. petiole node dorsally emarginated (petiole node dorsally not emarginated in *C. affinis*); in *C. periyarensis* sp. nov. propodeum with a pair of denticles (propodeum with a pair of distinct long spines in *C. affinis*); in *C. periyarensis* sp. nov. head finely and longitudinally striate throughout except small smooth median area at the frontal region, pronotum dorsally with longitudinal striations and laterally with transverse striations (head feebly longitudinally striate and mostly smooth with punctures, pronotum mostly smooth with few transverse striations anteriorly in *C. affinis*).

From *C. diversa* (Fig. 7 E, F), it can be distinguished by the following combination of characteristics; in *C. periyarensis* sp. nov. body size not large TL (Major worker 3.54-3.76mm and Minor Worker 1.76-1.92mm) (body of *C. diversa* is disproportionately huge TL (Major worker 10.34-11.71mm and Minor Worker 2.08-2.57mm); in *C. periyarensis* sp. nov. head is short without a median ocellus in front and propodeum with a pair of short spine (head massive and subtriangular with a median ocellus in front and propodeum with a pair of distinct long spines in *C. diversa*)


Figs 7. A. Head in full face view of *Carebara periyarensis* sp. nov. B. Profile view of *C. periyarensis*. C. Head in full face view of *C. affinis*. D. Profile view of *C. affinis*. E. Head in full face view of *C. diversa* sp. nov. F. Profile view of *C. diversa*.

Queen: Unknown

Male: Unknown

Bionomics: The workers were manually collected beneath a stone in Medaganam region of the Periyar Tiger Reserve. The ground is heavily covered with leaf litter and has a dense canopy overhead. The region has an average daily temperature of 32°C and is comprised of intact tropical wet evergreen forest with minimum light penetration.

Etymology: The species has been named after the name of the Periyar Tiger Reserve.

## ACKNOWLEDGEMENTS

Financial assistance rendered by the Ministry of Environment, Forest and Climate Change (Grant No. 22018/41/2010-CS (Tax)), Govt. of India, New Delhi is gratefully acknowledged. We also thank Forest and Wildlife Department, Govt. of Kerala for granting the permission to collect the research material vide Order No. WL 10-55389/2014 dated 07.01.2016.

### Identification key to the known species of genus *Carebara* from India

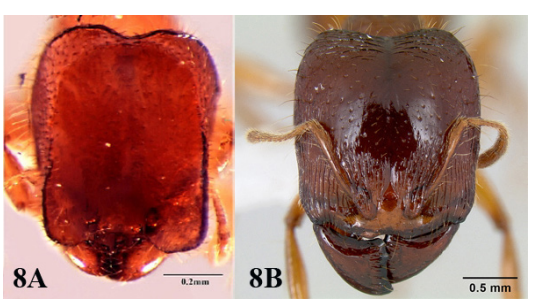
The identification key to the known species of genus *Carebara* from India is provided by Akbar and Bharti (2017). Since the new species mentioned here needs to be included, the following key couplets are slightly modified without changing the rest of the key.

1. Antenna 11-segmented ..... 2

- Antenna 9-segmented ..... 4

2. Head truncate, shield like, with frontal lobes extended forward forming broad rounded lamina; posterolateral edges sharply angulate (Fig. 8A)..... .....*C. nayana* (Sheela and Narendran)

- Head not truncate, sides straight to weakly convex; posterolateral corners rounded (Fig. 8B) ..…….....……...3




Figs. 8. A. Head in full face view of *C. nayana*; B. Head in full face view of *C. affinis*.

3a. Propodeum with a pair of distinct long spines (Fig. 9A)…….....3b

- Propodeum with a pair of denticles (Fig. 9B)……*Carebara periyarensis* sp. nov.


Figs 9. A. Profile view of *C. diversa* B. Profile view of *C. periyarensis* sp.nov.

3b. Head with a deep median longitudinal groove and a single ocellus, body strongly sculptured, relatively larger in size and dark reddish brown to black in color, (Fig. 10A) .....*C. diversa* (Jerdon)

-Head without deep median longitudinal groove and without ocellus, body weakly sculptured, relatively smaller in size and reddish brown in color (Fig. 10B) ……*C. affinis* (Jerdon)


Figs. 10. A. Head in full face view of *C. diversa* B. Head in full face view of *C. affinis.*

Note: From couplet 4 onwards there are no changes to the key presented by Akbar and Bharti (2017) and we refer to that publication.

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*J. Entomol. Res. Soc., 24(3): 417-427, 2022* *Research Article*
*Doi: 10.51963/jers.v24i3.2096* *Online ISSN:2651-3579*

# Museum Pests in Mammal Furs: An Investigation of the Correlation Between Pests and Host Furs

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## ABSTRACT

Insects are of great importance in the ecosystem. In some cases, however, they can be directly or indirectly harmful to humans. Pest insects in museums are good examples of such insects. Especially organic cultural heritage is often the target of these pests. To accomplish effective pest control, it is necessary to obtain knowledge on pest species and their effects. In this study, pest specimens including adults, larvae, pupae and exuviae materials (Total: 1323 specimens) were collected from 59 furs belonging to 12 species of mammals in Zoological Collection of University Istanbul (ZMUI). The species of these pests from various life stages were identified and the numbers were recorded. Further analyses were conducted through the means of Corresponding Analysis and a potential correlation between pest species and species of hosting furs was investigated 9 species of museum pests were identified; *Anthrenus verbasci* (Linnaeus, 1767), *Attagenus brunneus* Faldermann, 1835, *Dermestes maculatus* (DeGeer, 1774), *Lasioderma serricorne* (Fabricius, 1792), *Lepisma* sp., *Monopis* sp., *Ptinus clavipes* Panzer, 1792, *Stegobium paniceum* (Linnaeus, 1758) and *Tineola bisselliella* (Hummel, 1823). Among the pest species, *Anthrenus verbasci* is the species with the highest specimen count in the collection, as well as the most commonly distributed species. Museum pests are mainly observed on the furs of herbivore mammals. This observation is demonstrated with Corresponding Analysis. Also through Corresponding Analysis, a correlation between the species of pest insects and the diets of the species of hosting furs was demonstrated.

*Key words*: Museum Pests, Mammalian Furs, Corresponding Analysis, Turkey, ZMUI.

Özuluğ, O., Çakan, F.B., Erözen, A.A., & Kaya, N., (2022). Museum pests in mammal furs: An investigation of the correlation between pests and host furs. *Journal of the Entomological Research Society*, 24(3), 417-427.

Received: August 07, 2021 Accepted: November 30, 2022

## INTRODUCTION

For scientific studies, especially the studies on organisms such as plants or animals, it has been a very important step to collect specimens of different groups and preserve them in a suitable state for further studying. In this sense, it is crucial to preserve the collection materials in the museum, for as long as possible, against both abiotic factors like weather conditions and biotic factors, such as mold or museum pest insects. The success of insects in environmental adaptation and the breadth of their nutrient repertoire can result in humans encountering harmful (pest) insects in many areas. Natural history museums, herbariums and various animal collections are preferred places for settlement and spread for insects which naturally feed on dead tissues or debris.

Insects that are museum pests come from a variety of orders and families. While some gnaw through wooden material like drawers or display cases. Some of them directly feed on mammalian furs or bird feathers. They can damage these specimens in different levels of severity**,** from mild scratches to the point of losing the entire specimen. Thus, they pose a serious threat. Museum pests should be treated with serious effort and caution (Trematerra & Pinniger, 2018). In recent years, conservators and other museum staff have worked to develop alternative strategies for preventing and controlling pests. In the light of such studies, Integrated Pest Management (IPM) strategies were developed and adopted with success (Natural Sciences Collections Association, 2022; The Museum Pests Working Group (MPWG), 2022; Trematerra & Pinniger, 2018). In addition, a guide for pest control has been released in Turkey as well (Prevention of Pests in Written Work Collections, Ministry of Written Works Institution of Turkey, Department of Book Hospital and Archives, Research and Development Unit, 2022; Koçak & Eskici, 2019).

Many protocols and treatment methods are known to prevent or control these museum pest insects (Klein, 2008). These include applying pesticides, freezing the specimens, heat treatment, Anaxia, $CO_2$ or disinfecting with alcohol and such chemicals. However, an effective pest control can only be achieved if the appropriate methods are selected according to the specific types of insects. Because of that the first step in controlling museum pest insects is identifying the pest species that reside in the museum or collection. Knowing the species of pests gives us a lot of information about the life stages of the species, the period in which they feed on dead organic tissue (adults or larvae), what environmental conditions they prefer (temperature, humidity, etc.) and what are the times of spawning and hatching (bivalent or univalent). This information allows us to use their weakest points to fight these pests and to manage successful pest control. Furthermore, according to some publications, the species of pest insects may indicate various conditions of the collection environment (Notton, 2018). Thus, a correct identification of pest species also indicates what type of control method should be used or which environmental conditions should the material be stored in. In literature, many different publications may be found about museum pest insects that pose a danger to zoological collections (Suarez & Tsutsui, 2004; Klein,

2008; Pinniger, 2011; Querner, 2015; Notton, 2018; Trematerra & Pinniger, 2018). There are studies conducted on museum pests in Turkey (Koçak, & Eskici, 2019; Prevention of Pests in Written Work Collections, Ministry of Written Works Institution of Turkey, Department of Book Hospital and Archives, Research and Development Unit, 2022). However, none of these studies consider this situation for a natural history museum or a zoological collection. The goal of this paper is to identify the pest insect species found on mammalian furs and investigate if there is a correlation between the species of host mammal furs and pest insects.

In this research, pest insects found on mammalian furs in ZMUI (Zoological Museum of University Istanbul) were studied for their species identification and the existence of a specific fur preference of the pest insects was questioned. For this reason, the distribution of pest species on different species of mammalian furs were also analyzed in this study.

## MATERIAL AND METHODS

This study was conducted on the collection of mammalian furs in the Zoological Collection of İstanbul University in Vezneciler, İstanbul. The study holds the record of being the first study that is conducted on pest insects in a zoological collection in Turkey. The diversity of animals in the fur collection in question was largely provided by Prof. Dr. Curt Kosswig, who holds an important place in the history of research on the Anatolian fauna. He performed many comprehensive studies and student excursions and greatly contributed to the collection (İshakoğlu-Kadıoğlu, 1998; Küçüker et al, 2018).

Our study is conducted on 59 mammal furs belonging to following species: *Caracal caracal* (Schreber, 1776) (n=1)*, Capreolus capreolus* (Linnaeus, 1758) (n=2)*, Cervus elaphus* Linnaeus, 1758 (n=1)*, Felis catus* Linnaeus, 1758 (n=1)*, Herpestes ichneumon* (Linnaeus, 1758) (n=3), *Hystrix* sp. (n=2), *Lepus europaeus* (Pallas, 1778) (n=23)*, Lutra lutra* (Linnaeus, 1758) (n=4), *Martes foina* Erxleben, 1777 (n=2)*, Meles meles* Linnaeus, 1758 (n=13)*, Sus scrofa* (Linnaeus, 1758) (n=1)*, Vulpes vulpes* (Linnaeus, 1758) (n=6).

These furs were collected from Anatolia and Thrace between 1940-1970 through various student excursions or as research projects for students. Furs are known to be prepared with various tanning methods, however, there are no records that indicate which chemical procedure is used for preparation. After tanning, furs of the same species were stored together in wooden closets. Each fur was wrapped in raw cotton fabric and has been stored that way since 2000. During the insect sampling, specimens found on the fur or between its hairs, in which they were most abundant, were collected with sieves and placed in lidded plastic containers. The containers were tagged according to which species of furs its contents were collected from. After collection, the containers were brought to the lab and each one of the pest specimens were inspected under stereozoom microscope and their species were identified. Species identification was carried out according to following publications: Jackson (1906), Bousquet (1990), Gorham (1991), Choe (2013), Hackston (2014) &

Notton (2018). Pictures of pest insects were taken with a Canon D600 camera under a Leica stereozoom microscope.

The data is recorded on Microsoft Excel, then used for creating tables and figures for better interpretation. Also with this data, Correspondence Analysis (CA) was used to analyze the correlation between museum pests and the host furs by the Past software 4.05 (Hammer et al, 2001).

## RESULTS

Out of 1323 examined pest insects: 54 adults, 50 larvae, 1112 exuviae and 88 cocoons were found and investigated. In addition, 19 recognizable carcass remnants were identified and included in the study. *Attagenus brunneus*, *Anthrenus verbasci*, *Dermestes maculatus*, *Lasioderma serricorne*, *Lepisma* sp., *Monopis* sp., *Ptinus clavipes*, *Stegobium paniceum* and *Tineola bisselliella* are identified. The taxonomic details are as given below.

**Order: Coleoptera**

**Family: Dermestidae**

*Attagenus brunneus* Faldermann, 1835 (Brown-Black Carpet Beetle - Fig. 1) Adult, 1; Larva, 3; Exuvia, 68; Pupa, 0; Carcass, 2.

*Anthrenus verbasci* (Linnaeus, 1767) (Varied Carpet Beetle - Fig. 2) Adult, 33; Larva, 41; Exuvia, 968; Pupa, 0; Carcass, 13.

*Dermestes maculatus* (DeGeer, 1774) (Fur Beetle - Fig. 3) Adult, 9; Larva: 6; Exuvia, 76; Pupa, 0; Carcass, 4.

**Family: Anobiidae**

*Lasioderma serricorne* (Fabricius, 1792) (Cigarette Beetle - Fig. 4) Adult, 1; Larva, 0; Exuvia, 0; Pupa, 0; Carcass, 0.

*Stegobium paniceum* (Linnaeus, 1758) (Drugstore Beetle - Fig. 5) Adult, 3; Larva, 0; Exuvia, 0; Pupa, 0; Carcass, 0.

**Family: Ptinidae**

*Ptinus clavipes* Panzer, 1792 (Spider Beetle - Fig. 6) Adult, 2; Larva, 0; Exuvia, 0; Pupa, 0; Carcass, 0.

**Order: Lepidoptera**

**Family: Tineidae**

*Monopis cf crocicapitella*. Only 1 individual is found. This individual does not possess enough characteristic information for definitive species identification. No other larvae, pupae or exuviae belonging to this species were found. This individual is thus reported here but disregarded from calculations and analyses.

*Tineola bisselliella* (Hummel, 1823) (Webbing Clothes Moth - Fig. 7) Adult, 1; Larva, 0; Exuvia, 0; Pupa, 88; Carcass, 0.

## Order: Zygentoma

### Family: Lepismatidae

*Lepisma* sp. Adult, 3; Larva, 0; Exuvia, 0; Pupa, 0; Carcass, 0.

These individuals were identified as adult *Lepisma* sp. However, they do not possess important characteristic information for species identification.

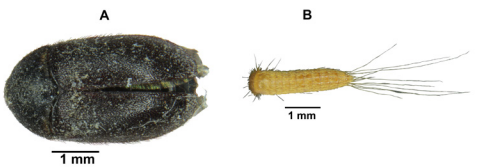


Fig. 1. Dorsal view of *Attagenus brunneus,* adult (A) and larva (B).

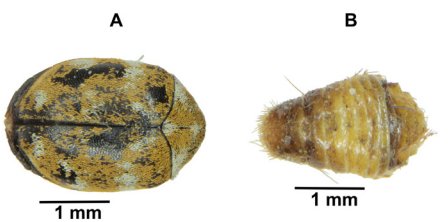


Fig. 2. Dorsal view of *Anthrenus verbasci* species, adult (A) and larva (B).


Fig. 3. Dorsal view of *Dermestes maculatus,* adult (A) and larva (B).


Fig. 4. Dorsal view of *Lasioderma serricorne,* adult.


Fig. 5. Dorsal view of *Stegobium paniceum,* adult.


Fig. 6. Dorsal view of *Ptinus clavipes,* female (A) and male (B) adults.

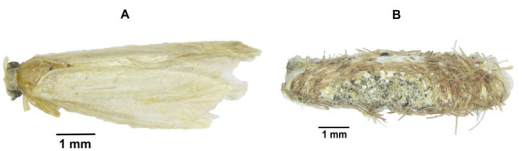


Fig. 7. Dorsal view of *Tineola bisselliella,* A. adult B. pupa.

Table shows how many of each pest species were found on each fur (Table 1). These numbers are the sum of all life stages of the pest species (adult, larva, exuvia, pupa, carcass). *A. verbasci* (total no: 1055) has the highest individual numbers in terms of adults, larvae and exuviae. In addition, it was identified on 11 of the 12 species of mammal furs. *L. serricorne* has the lowest individual number. It was found on one fur belong to *L. europaeus.* Count of different life stages of different pest species are showed in Fig. 8 and the distribution and density of pests on host furs are showed in Fig. 9. *A. verbasci* has the highest count of exuviae and larvae. *T. bisselliella* has the highest count of pupae. The correlation between museum pests and the host furs was given by Correspondence Analysis (CA) (Fig. 10). According to CA, mammal species of *C. elaphus, L. europaeus, Hystrix* sp., *S. scrofa* and *M. meles* have high correlations with the pest species. In contrast, *M. foina* and *H. ichneumon* have lower correlations.

Table 1. Species and numbers of furs and the pests found on them.

| Host / Pest | Number of furs in the collection | *Attagenus brunneus* | *Anthrenus verbasci* | *Dermestes maculatus* | *Lasioderma serricorne* | *Lepisma sp.* | *Ptinus clavipes* | *Stegobium paniceum* | *Tineola bisselliella* | Total number of species |
|---|---|---|---|---|---|---|---|---|---|---|
| *Caracal caracal* | 1 | | 1 | 1 | | | | | | 2 |
| *Capreolus capreolus* | 2 | | 20 | | | | | | 25 | 3 |
| *Cervus elaphus* | 1 | 1 | 41 | | | | | | | 2 |
| *Felis catus* | 1 | 5 | 15 | 27 | | | | | 1 | 4 |
| *Herpestes ichneumon* | 3 | | 1 | 5 | | | | | | 2 |
| *Hystrix* sp. | 2 | 2 | 8 | | | | 1 | 1 | 1 | 5 |
| *Lepus europaeus* | 23 | 33 | 774 | 1 | 1 | 3 | 1 | 1 | 7 | 8 |
| *Lutra lutra* | 4 | 4 | 7 | 2 | | | | | | 3 |
| *Martes foina* | 2 | | | 1 | | | | | | 1 |
| *Meles meles* | 13 | 19 | 84 | 30 | | | | 1 | 32 | 5 |
| *Sus scrofa* | 1 | 10 | 73 | 10 | | | | | 11 | 4 |
| *Vulpes vulpes* | 6 | | 31 | 18 | | | | | 12 | 3 |
| Total specimen count | 59 | 74 | 1055 | 95 | 1 | 3 | 2 | 3 | 89 | |

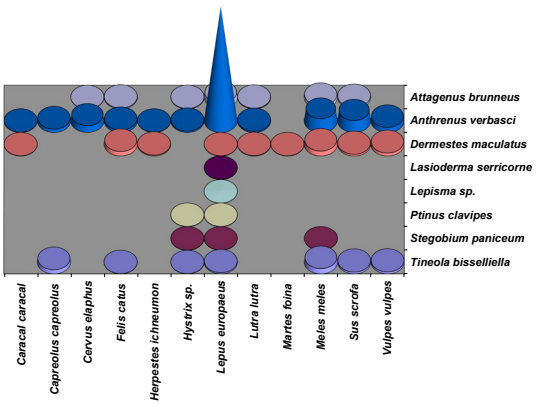


Fig. 8. Count of different life stages of different pest species.

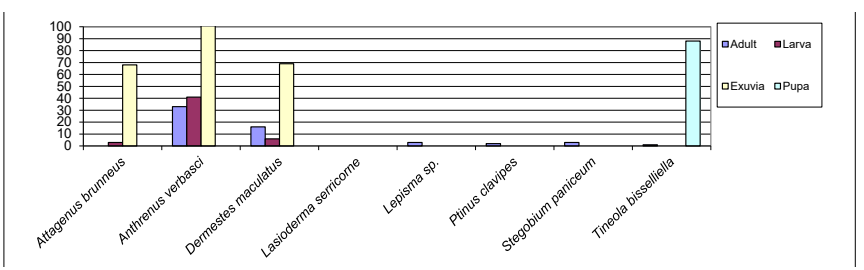


Fig. 9. The distribution and density of pests on host furs.

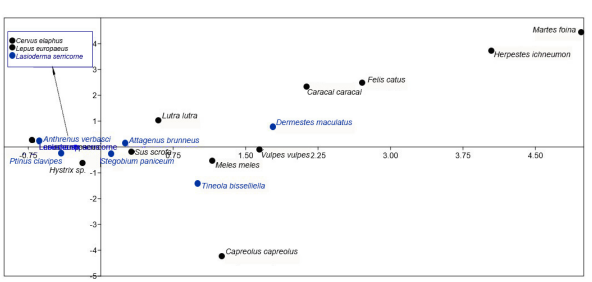


Fig. 10. The correlation between hosts and pests are shown through Corresponding Analysis (CA).

## DISCUSSION

In our study, a total of 59 furs, belonging to 12 mammal species from the zoological collection were examined. All pests found on these furs are recorded by their species and number of individuals per species (Table 1). Results of these examinations show that total individual numbers in terms of adults, larvae and exuviae are the highest for *A. verbasci* (total count: 1055) compared to other pest species (Fig. 8). The natural habitat of this insect, which is also known as varied carpet beetle, is originally dried bird nests (Nisimura & Numata 2003). However, it is recorded as a pest species in

museums and there are many publications about its life cycle and control methods (Nisimura & Numata 2003; Kumar et al, 2013).

*A. verbasci* is a 2-3 mm beetle, which is a cosmopolitan species, being recorded throughout Europe, Africa, America, Asia and Australia (Tezcan et al, 2004). As seen in Fig. 9, *A. verbasci* is the most abundant pest species in the collection. It was found on 11 of the 12 species of furs, only absent from the fur of beech marten. As this species is a widespread, dominant pest, it can be said that our findings are in line with previous studies (Yıldırım, 2013).

The second most abundant pest in the collection is *D. maculatus* (total count: 95) (Fig. 8). Many species in the genus *Dermestes* are known to be pests. *Dermestes* sp. are an invasive group of pests that originated from Asia and introduced to Europe later on with human activity and are known to be a capable of dealing considerable economical damage (Manachini, 2015). It was found on 9 of the 12 species of furs in the collection with a total number of 95 (Fig. 9). When compared to *A. verbasci* in terms of total individual counts, *D. maculatus* is seen to have 10 times less potential for distribution. On the other hand, *D. maculatus* is the only pest species that was found on the fur of beech marten.

The pest with the third highest number of individuals is *T. bisselliella* (total count: 89) (Table 1.). *T. bisselliella* is found on 7 of the 12 species of furs in our study (Fig. 9). This species has the highest number of pupae (Fig. 8). According to Choe (2013), adult individuals of *T. bisselliella* do not share the same food source with the larvae. In reference to this information, it can be said that the pests that are actually harming the collection in *T. bisselliella*'s case are only the larvae. This explanation also further supported by the fact that only 1 adult is found on the furs as opposed to the very high count of pupae. The absence of *T. bisselliella* adults on the furs appears to be because the adults do not use the fur as a source of food. These findings also appear to be in line with previous studies.

Another pest species, *A. brunneus,* has a high count of exuviae and larvae but low count of adults (Fig. 8). As the adult stage of this beetle life cycle is as low as 2 weeks, while the larva stage is around 2 years, this difference between the count of life stages also seems to be expectable (Story, 1985).

In our study, 4 out of 9 pest species (*A. verbasci*, *D. maculatus, T. bisselliella, A. brunneus*) were observed to have high numbers as dominant species, compared to the rest. One of the pest species found in low numbers is *P. clavipes*. There were found to be only 2 adult individuals of this species, one of which being male and other one being female (Fig. 6). This is a species that had only been recorded from Turkey once before, from another city (Uşak) and not from a museum, but from a wheat storehouse (Zengin & Karaca, 2019). In that paper, there is no description or figures to represent this species. It is important to note that *Ptinus clavipes* is morphologically similar to *Ptinus tectus*. Therefore, the characteristics used for the species identification is given in this paper. The specimen in this study demonstrates the clear identification character to tell the two species apart: vestiture of scutellum is denser and whiter in color than rest of the elytra, as can be seen in Fig. 6 (Bousquet, 1990; Gorham, 1991). Also,

the species *P. clavipes* is reported as a first time record from İstanbul, as it was not mentioned in the previous study, also conducted in Istanbul (Dikmen & Özuluğ, 2018).

The investigation of correlation between pests and host furs shows that the fur with the most pest diversity is *L. europaeus*. All the pest species inhabited these furs (Fig. 9). The fact that the number of hare furs (23) is higher when compared to other species of furs contributed both to the diversity and total number of pests found on it.

The total number of different pest species found on hare furs (8 different species) are also higher than other mammal furs (Table 1., Fig. 9). The fur species observed to have the least diversity of pests is *M. foina* (Table 1., Fig. 9). Furs of beech marten are also the only furs that the most abundant pest, *A. verbasci* is absent from. The fact that this widespread pest is absent from these furs is crucial. The pest species found on furs of *M. foina* is *D. maculatus*. *D. maculatus* is found on 9 out of 12 species of furs.

The Correspondence Analysis demonstrates four important points. First one is the (0) point at where the horizontal and vertical axes intersect. At this central point, it can be seen that the pest species *A. verbasci, L. serricorne, Lepisma* sp., *P. clavipes* and *S. paniceum* are in close relation with the furs of *Lepus europaeus, Cervus elaphus* and *Hystrix* sp. In other words, the furs which are infested the most by pests are European hare, red deer and porcupine furs. These animals, which pests are mostly on, are herbivores. The second grouping in the analysis shows that *A. brunneus* is in close relation with *L. lutra* and *S. scrofa* furs. Both the common otter and the wild boar are omnivores. In the third group, the relation between the pest *T. bisselliella* and the fur of *M. meles* is demonstrated. *C. capreolus* also groups with *T. bisselliella,* but it can also be seen that this is the most distant fur in relation to other furs. This is the result of the fact that *T. bisselliella* is the only pest to infest *C. capreolus* (Roe deer). Honey badger is an omnivore whereas the roe deer is an herbivore. In the fourth grouping, the close relation between the pest *D. maculatus* and the furs of *C. caracal* and *V. vulpes* can be seen, while the furs of *H. ichneumon* and *M. foina* show distant relation with the pests. These animals are carnivores (Fig. 10).

High humidity and a temperature of 18-25ºC is known to provide a good environment for development of pests (Richardson & Goff, 2001). Given the fact that all of the furs had been under the same environmental conditions; the difference of distribution and abundance between the 4 most abundant pest species (*A. brunneus, A. verbasci*, *D. maculatus, T. bisselliella*) and the rest of them (*L. serricorne*, *Lepisma* sp., *P. clavipes*, *S. paniceum*) suggests that even if the conditions are favorable, not all pests would be found on all species of furs.

According to Querner (2015), species of the genus *Ptinus* prefer museum materials that are of plant origin. Thus, the fact that *P. clavipes* is found on an animal fur in this study is surprising. However, as explained above, groupings of the Corresponding Analysis (Fig. 10) suggest that there may be a noticeable fur preference of pest insects, according to the diets (herbivore, carnivore and omnivore) of the mammalian furs. Given these findings, and the fact that *P. clavipes* was discovered on the fur of an herbivore animal, it appears reasonable to expect *P. clavipes* to be found on the furs of herbivore animals in museums. Physical properties of the furs can be considered

among the reasons for the grouping observed in the results of CA analysis. These physical characteristics may include the hardness or softness of the skin and hair of the mammalian fur, as well as the density or sparsity of the hairs. Many factors, such as these, could have contributed to the uneven distribution of pests in mammalian furs. Evaluating or proving these possibilities is not within the scope of our study and may be the subject of future research. However, this study discovered a relationship between the species of infested furs and the pest insects that infest them.

## CONCLUSION

In conclusion, in our study, which is conducted on mammalian furs in the zoological collection of Istanbul University, insects that are known to be museum pests were identified and their distribution in terms of the animal furs is put forward. We discovered a potential preference of the pest insects for mammalian furs. One of the species of museum pests, *Ptinus clavipes,* is reported as a first time record from İstanbul, as well as a first time record from a museum in Turkey.

## ACKNOWLEDGEMENTS

This study was funded by Scientific Research Projects Coordination Unit of Istanbul University. Project number: FLO-2018-32194. We would also like to thank Dr. Mustafa Kılıç and Esra Albayrak for their help in the collection process.

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Material examined: Ankara, Altındağ, Çubuk Dam Lake, 900 m, 29.06.1998, 1 ♂; Kalecik, 600 m, 24. 07. 2001, 2 ♀♀, Kalecik, 800 m, 25. 07. 2001, 3 ♀♀
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