

ONLINE ISSN 2651-3579



***Journal
of the Entomological Research
Society***

Volume: 26

Part: 1

2024

JOURNAL OF THE ENTOMOLOGICAL RESEARCH SOCIETY

Published by the Gazi Entomological Research Society

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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: March 28, 2024

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Insecticidal Activity of Essential Oils Derived from Lavender, Laurel and Peppermint Against Lesser Grain Borer, *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae)

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ABSTRACT

Fumigant effects of the essential oils from *Laurus nobilis* L., 1753 (Laurales: Lauraceae), *Mentha piperita* L., 1753 and *Lavandula x intermedia* Emeric ex Loisel, 1828 (Lamiales: Lamiaceae) were determined against the lesser grain borer [*Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae)], an important pest species of stored grains.

Essential oils were extracted by steam distillation by Clevenger apparatus and analyzed by capillary gas chromatography-mass spectrometry. The major compounds of the essential oils were detected as linalool (43.33%), 1,8-Cineole (57.67%) and menthone (53.51%) in *Lavandula x intermedia*, *L. nobilis* and *M. piperita*, respectively. The experiments were conducted in a controlled-climate chamber at 27 ± 1 °C, 50 ± 10% RH and in the dark. The fumigant effects of essential oils were determined at five concentrations (1, 5, 20, 50, and 200 µl/L air) with larval, pupal, and adult stages of *R. dominica*. The highest concentrations of *L. nobilis* and *M. piperita* essential oils completely killed the adults of *R. dominica*, while *L. nobilis* killed 100% of the larval stage of the pest. However, the pupal stage was less susceptible, in which the highest mortality was 49% with *L. nobilis* essential oil. As a result, it is considered that both *L. nobilis* and *M. piperita* essential oils have the potential to be used as effective biofumigants for stored product pests.

Keywords: Essential oil, fumigant, *Laurus nobilis*, *Lavandula intermedia*, *Mentha piperita*

Çilgin, E. & Keçeci, M. (2024). Insecticidal activity of essential oils derived from lavender, laurel and peppermint against lesser grain borer, *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae). *Journal of the Entomological Research Society*, 26(1), 1-16.

Received: March 13, 2023

Accepted: November 18, 2023

INTRODUCTION

Cultural, mechanical and chemical methods are employed to prevent contamination and eliminate potential damage for controlling stored product pests. In Türkiye, fumigation is common control method for *Rhyzopertha dominica*, (Fabricius, 1792) (Coleoptera: Bostrichidae), which is similar to the control of many other pests active in stored products.

Chemical control methods in stored product pests can lead to negative effects for humans, animals, plants and other organisms, and troubles affiliated with the occurrence of pest resistance, residual toxicity, and the demolition of natural balance. There is worldwide worry about the prejudicial effects of fumigants and synthetic insecticides, including as depletion of the ozone layer, ecological pollution, toxicity on non-target organisms and pest resistance (Ogendo et al., 2008; E. Shaaya, Kostjukovski, Eilberg, & Sukprakarn, 1997; Kalpna, Hajam, & Kumar, 2022; Ngegba, Cui, Khalid, & Zhong, 2022). Due to prevalent use of synthetic chemical insecticides, insects develop resistance, complicating pest control (Whalon, Mota-Sanchez, & Hollingworth, 2008). It has been revealed that several pests of stored product can develop resistance to synthetic pyrethroid and organophosphorus insecticides (Attia et al., 2020; Collins, Lambkin, Bridgeman, & Pulvirenti, 1993; Lee & Lees, 2001; Subramanyam, Harein, & Cutkomp, 1989; Zettler & Cuperus, 1990). Especially after the methyl bromide ban, phosphine gas has been widely utilized as a fumigant against stored product pests. In addition to mistakes made during application of this gas, the employment of phosphine gas as a single control method for several years led to the development of resistance by stored product pests. The coleopter pests *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) (Opit, Phillips, Aikins, & Hasan, 2012; Pimentel, Faroni, Tótolá, & Guedes, 2007; Pimentel, Faroni, Batista, & Silva, 2008; Wakil, Kavallieratos, Usman, Gulzar, & El-Shafie, 2021), *Sitophilus granarius* (Linnaeus, 1758) (Wakil et al., 2021), *Sitophilus oryzae* (Linnaeus, 1763) (Holloway, Falk, Emery, Collins, & Nayak, 2016), *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) (Pimentel et al., 2008), *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae) (Pimentel et al., 2007), *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) (Wakil et al., 2021), *Lasioderma serricorne* (Fabricius, 1792) (Coleoptera: Anobiidae) (Baliota, Athanassiou, & Cohnstaedt, 2021; Sağlam, Edde, & Phillips, 2015) have been stated to have evolved resistance to phosphine gas. Similarly, other studies reported that *R. dominica* has also developed resistant to phosphine gas (Afful, Elliott, Nayak, & Phillips, 2018; Opit et al., 2012; Pimentel et al., 2007; Pimentel et al., 2008; Wakil et al., 2021).

Due to the increasing difficulties in pesticide resistance management, alternatives are required to control the pests in storage of new and environment friendly products (Duke et al., 2003). The share of herbal extracts and essential oils (EOs) is significant among alternative control methods and these products have a potential in the protection of agricultural crops. There is estimated to be 17 500 aromatic plant species in nature (Bruneton, 1995), and 3 000 of these species are known to include EOs, and over 300 plant EOs are commercially available in medicine, cosmetics and perfumery industries

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(Franzios et al., 1997), in addition to the pesticide industry (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). EOs exhibit fumigant effects and contact toxicity (Abdelgaleil, Mohamed, Badawy, & El-arami, 2009; Alkan, 2020; Baş & Ersoy, 2020; Göktürk, Kordali, Ak, Kesdek, & Bozhüyük, 2020; Sahaf, Moharramipour, & Meshkatalsadat, 2008), repellency (Alkan, 2020; Alkan et al., 2021; Nerio, Olivero-Verbel, & Stashenko, 2009; Wang, Zhu, Zhou, Niu, & Lei, 2006), and can inhibit feeding (Huang, Tan, Kini, & Ho, 1997) growth (Tomova, Waterhouse, & Doberski, 2005; Waliwitiya, Kennedy, & Lowenberger, 2009) and sublethal effects (such as decreasing digestive enzyme activities; reduction in protein, lipid, and carbohydrate level; low consumption rate or etc. (Ebadollahi, Naseri, Abedi, & Setzer, 2022; Ebadollahi, Naseri, Abedi, Setzer, & Changbunjong, 2022; Ebadollahi, Ziaee, & Palla, 2020) when used in the control of insect pests.

The plants from which these essential oils come are mostly in the families Lamiaceae, Lauraceae, Asteraceae, Cupressaceae, Myrtaceae, Rutaceae, Piperaceae and Poaceae (Campolo, Giunti, Russo, Palmeri, & Zappalà, 2018). The relative constituent of essential oils varies considerably and depends on geographical location, climatic conditions and various other factors. There are many studies related to the essential oil's chemical composition. Previous studies reported 1,8-cineole 46.5 % (Baratta, Dorman, Deans, Biondi, & Ruberto, 1998) and 41.9 % (Simić et al., 2004) as the main constituents of *L. nobilis*. However, the same compound was reported as 24.55 % in the plant cultivated in Tunisia (Mediouni Ben Jemâa, Tersim, Toudert, & Khouja, 2012). Although menthol was the main compound (37.3%) in *M. piperita* collected from Amazonia, Brasil (Miura et al., 2021), it was not high (24.71%) as much as from Ardabil province, moreover main constituents was limonene (27.28%) (Ebadollahi, Davari, Razmjou, & Naseri, 2017). It is therefore assumed that the insecticidal effect of the essential oils of plants that grow in different regions could be significant.

Several studies have reported effects of herbal EOs on stored product pests. However, studies on fumigants for *R. dominica* did not target larval or pupal stages of the pest. Thus, the present study aimed to assess the fumigant toxicity of EOs extracted from three plants for controlling of *R. dominica* larvae, pupa and adults, given this species is a significant stored product pest.

MATERIALS AND METHODS

The experiments were conducted in the 2021 in the Plant Protection Department, Agricultural Faculty, Malatya Turgut Özal University, Türkiye.

Plant essential oils extraction

Lavandula x intermedia Emerica ex Loisel, 1828 (Lamiales: Lamiaceae) collected from Muratpaşa district of Antalya province and *Laurus nobilis* L., 1753 (Lurales: Lauraceae) collected from Anamur district of Mersin province were extracted from the Bati Akdeniz Agricultural Research Institute. *Mentha piperita* L., 1753 (Lamiales: Lamiaceae) collected from Ovacik district of Tunceli province was extracted from

Malatya Turgut Özal University, Faculty of Agriculture. Both EOs were extracted by steam distillation for 3 h by Clevenger apparatus. The EOs were kept in tightly closed glass tubes at 4 °C until used.

Essential oils content

Essential oil analyzes were conducted at the Batı Akdeniz Agricultural Research Institute. *Lavandula x intermedia*, *L. nobilis* and *M. piperita* EOs (diluted by 1:100 hexane) were characterized by GC/GC-MS (gas chromatography (Agilent 7890A) and mass detector (Agilent 5975C)) with a capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm). Helium gas was used as a carrier at a constant flow rate of 0.8 ml/min, and 1 µl samples were applied by injection with a split ratio of 40:1. The injector temperature was maintained at 250°C, and the column temperature was set to 60°C (10 min), increased from 60 to 220°C at a rate of 4°C/min and set again to 220°C (10 min). The total analysis duration was 60 min. The scanning range (m/z) was 35-450 atomic mass units and 70 electron volt (eV) electron bombardment ionization was used. The essential oil components were identified based on the data provided by Wiley and Adams oil libraries by comparison of their RT. The component rates were determined with the FID detector and the components were identified with the MS detector (Topuz et al., 2016; Gölükcü, Toker, Tokgöz, & Çınar, 2016; Syraiyl, Ydyrys, Ahmet, Aitbekov, & Imanaliyeva, 2022).

Insect rearing

Rhyzopertha dominica adults were procured from Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Plant Protection Department Entomology Laboratory. The adults were placed in 200 g wheat flour for 2-3 days to oviposit eggs, and then separated from the flour using a 500 µm sieve. To separate the eggs, the flour was sieved through a 212 µm sieve. The eggs were incubated in 500 g bread wheat grain in 1 L plastic jars with a gauze lid. Rearing was conducted in a dark controlled-climate chamber at 27 ± 1°C and 50 ± 10% RH (Cinco-Moroyoqui, Rosas-Burgos, Borboa-Flores, & Cortez-Rocha, 2006; Chanbang, Arthur, Wilde, & Throne, 2007).

Fumigant tests

EOs were tested at 1, 5, 20, 50, and 200 µl/L. An insecticide, dimethoate (50 µl/L) (Poligor EC) (Hektaş Ticaret Türk A.Ş.) was used as the comparator, since there is no available effective fumigant that could be practically applied in small volumes. Tests with no treatment were used as a negative control. The tests were conducted in a 135 ml autoclave bottle with a screw cap containing 10 g wheat grain. Filter paper was stuck with silicon adhesive to the inside of the caps, and the filter paper was impregnated with pure EOs with a micropipette.

Fumigant tests with *Rhyzopertha dominica* larvae

Fumigant tests were conducted with third instar larvae of *R. dominica*. To obtain third instar larvae, the eggs (without counting) obtained from 8,000-10,000 pests for 3 days were placed in plastic jars containing 500 g wheat grain. Edde (2012) reported that at

28°C, the egg stage lasted 7 days, first immature stage 9 days, second immature stage 6 days and third immature stage 5 days. Twenty-five to 27 days after the eggs were cultured, 25 g wheat grain, assumed to include third stage larvae, was transferred into autoclave bottles and 1, 5, 20, 50 or 200 µl/L essential oil was applied to filter paper in the autoclave bottle caps. *Rhyzopertha dominica* larvae were exposed to the EOs for 48 h at 27°C. The bottle lids were then removed, and the wheat was ventilated, and the contents were transferred into other plastic containers (with a tulle lid) and incubated at 27°C until adult emergence. The pest adults that emerged 5 weeks after the application were separated from the wheat grain with a sieve and counted. The adults that remained in the grain were collected after a further week. The trial was set up in a randomized plot design with five replicates.

Fumigant tests with *Rhyzopertha dominica* pupae

To determine the fumigant effects on *R. dominica* pupae, eggs were placed in plastic jars containing 500 g wheat grain. Forty to 42 days after the addition of eggs, 25 g wheat grain, assumed to contain pest pupae, was transferred to autoclave bottles. One, 5, 20, 50 or 200 µl/L of essential oil was applied the filter paper in the bottle caps and *R. dominica* pupae were exposed for 48 h at 27°C. The wheat was then aerated, transferred to plastic containers with gauze lids and incubated at 27°C until adult emergence. Adults that emerged 3 weeks after treatment were separated from the wheat grain with a sieve and counted. The adults that remained in the grain were collected after a further week. The assessment was set up in a randomized plot design with five replicates.

Fumigant tests with *Rhyzopertha dominica* adults

Twenty mixed-sex *R. dominica* adults (1 to 10 days old) were placed in autoclave bottles containing 10 g wheat grain. Essential oil was applied the filter paper in the bottle caps and the bottles were tightly closed. The adults were exposed to EOs for 24 and 48 h at 27°C. The viability of the adults was then determined. The trial was set up in a randomized plot design with five replicates.

Data analysis

The adult mortality rate was corrected with the Abbott (1925) formula based on the counts conducted 5-6 weeks after the essential oil application in the larval test and 3-4 weeks after the application in the pupal test. The data on the effect of fumigation with essential oil on adult pests were also corrected using Abbott (1925) formula. Percentage mortality data then converted to an arcsine transformation. They were subjected to a one-way analysis (ANOVA). The differences between the mean values were determined using the Tukey test at the 5% significance level. The analysis of variance and the multiple comparison tests were performed using the SPSS program.

Dose response modeling was performed with *drc* function in the R package “drc” (Ritz, Baty, Streibig, & Gerhard, 2015; Ritz & Streibig, 2005) in R version 4.2.0. The fumigant essential oil concentration in the treatments were log transformed using

Equation (1). Then, essential oil concentration causing 50% *R. dominica* mortality (LC_{50}) in the treatments were obtained using the single three-parameter log-logistic regression model LL.3 (Equation 2) for each essential oil (Arena, Merlo, Defagó, & Zygadlo, 2020; Taquet et al., 2020).

$$x = \text{Log}10(\text{Fumigant concentration} + 0.1) \quad (1)$$

$$U = \frac{d}{1 + \left(\frac{x}{EC_{50}}\right)^b} \quad (2)$$

where, b is the slope, d the upper-limit determined by the fitted concentration-response model with lower limit fixed at 0 (3 parameters), and U the response.

RESULTS

Compositions of the essential oils employed in the trials

The chemical composition of the EOs examined is presented in Tables 1. Twenty-six components were determined in the *L. intermedia* essential oil with the main components were linalool (43.3%), linalyl acetate (19.8%), camphor (6.7%), and 1,8-cineole (4.4%) (Table 1). Nineteen components were characterized for the *L. nobilis* essential oil with the main constituents were 1,8-cineole (57.7%), alpha-terpinyl acetate (9.92%), and alpha-pinene (6.4%) (Table 1). Twenty-one components were detected in the *M. piperita* essential oil with the main constituents were menthone (53.5%), menthol (17.2%) and menthofuran (5.4%) (Table 1).

Table 1. Chemical composition of *Lavandula x intermedia*, *Laurus nobilis* and *Mentha piperita* essential oils

Compound	percentage (%)	Compound	percentage (%)
<i>Lavandula x intermedia</i>			
α -Bisabolol	0.84	Lavandulyl acetate	1.91
Borneol	2.38	Limonene	1.08
β -Caryophyllene	0.63	Linalool	43.33
Camphene	0.32	Linalyl acetate	19.82
Camphor	6.66	β -Myrcene	1.44
1,8-Cineole	4.38	Nerol	0.89
β -Citronellol	0.59	Neryl acetate	0.98
Cryptone	0.42	<i>cis</i> -Ocimene	1.76
Geraniol	2.18	<i>trans</i> -Ocimene	2.03
Geranyl acetate	1.79	3-Octanone	0.92
Germacrene-D	0.25	Octen-1-ol, acetate	0.34
1-Hexyl acetate	0.54	α -Terpineol	3.73
Hexyl butyrate	0.39	α -Terpinolene	0.40
<i>Laurus nobilis</i>			
Camphene	0.33	beta-Pinene	4.56
1,8-Cineole	57.67	Sabinene	4.47
Eugenol	0.33	<i>gamma</i> -Terpinene	2.10
<i>para</i> -Cymene	2.19	Terpinen-4-ol	4.67
Linalool	0.88	Terpinolene	0.58

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Table continued

Compound	percentage (%)	Compound	percentage (%)
Limonene	2.24	<i>alpha</i> -Terpineol	0.38
Methyl eugenol	0.54	<i>alpha</i> -Terpinyl acetate	9.92
Myrcene	0.48	<i>delta</i> -Terpinyl acetate	0.37
<i>alpha</i> -Phellandrene	1.19	<i>alpha</i> -Thujene	0.70
<i>alpha</i> -Pinene	6.40		
<i>Mentha piperita</i>			
β -Caryophyllene	0.89	β -Pinene	1.07
1,8-Cineole	4.61	Sabinene	0.59
Germacrene	0.80	neoisomenthyl acetate	0.28
Isomenthol	5.22	β -Ocimene	0.33
Limonene	1.30	3-Octanol	0.41
Linalool	0.30	α -Pinene	0.75
Menthol	17.21	Piperitone	0.89
Menthofuran	5.43	Pulegone	3.99
Menthone	53.51	<i>cis</i> -Sabinene hydrate	0.35
Menthyl acetate	1.10	Terpinen-4-ol	0.21
β -Myrcene	0.76		

The fumigant effects of the essential oils on the *Rhyzopertha dominica*

The mortality effect of the essential oils on different stages of *R. dominica* is shown in Fig. 1. The toxicity of the fumigant increased progressively with increasing concentration of the essential oils in the larval, pupal and adult stages. *Laurus nobilis* essential oils applied to the larvae of *R. dominica* had a very strong fumigant activity (Fig. 1a). At a dosage of 200 μ l/L, larval mortality reached 100% and was statistically different from the other plant oils ($F_{15,79}=65.717$, $P=0.000$). At the same dose, the larval mortality of *M. piperita* was 59.04%.

The larvae and adults of *R. dominica* were more susceptible than the pupal stages of the pest. None of the applied concentrations or the standard toxic insecticide dimethoate caused 100% mortality. However, the highest mortality (%49) was achieved by the highest dose of *L. nobilis* and was significantly different from all *L. intermedia* doses ($F_{15,79}=4.298$, $P=0.000$) (Fig. 1b).

Compared to *L. intermedia*, *M. piperita* and *L. nobilis* caused higher mortality in adult *R. dominica* at each dose (Fig. 1c-d). At a dose of 200 μ l/L, both *M. piperita* and *L. nobilis* caused the highest adult mortality of *R. dominica*, while *L. intermedia* caused significantly low (54%) adult mortality at 24-h exposure ($F_{15,79}=44.369$ $P=0.000$). It was similar in the 48-hour exposure experiment. At a dose of 50 μ l/L, the highest adult mortality was achieved by *L. nobilis* (72%), followed by *M. piperita* (38%) and *L. intermedia* (15%) ($F_{15,79}=48.404$, $P=0.000$).

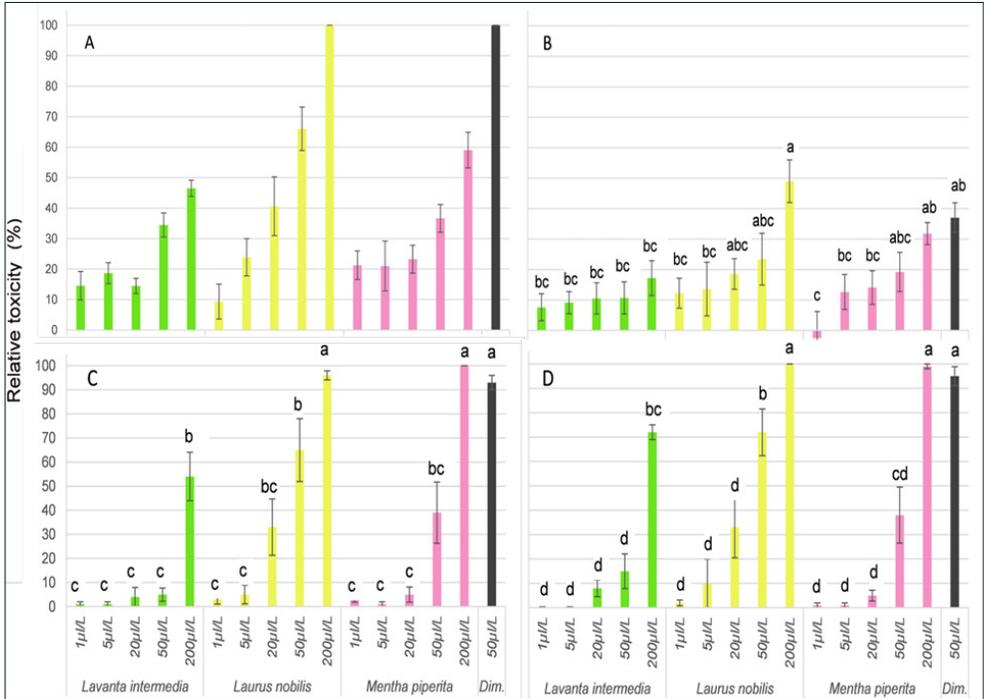


Figure 1. Fumigant effect of essential oils on the mortality of different *Rhyzopertha dominica* stages; A) larva, B) pupa, C) adult (24h exposure), D) adult (48h exposure) (different lower-case letters show statistical differences according to Tukey test at $P \leq 0.05$; bars represent standard errors; Dim.: Dimethoate).

The three-parameter log-logistic models, as presented in Equation 2, fitted to the data are as shown in Fig. 2. This demonstrated that the essential oil concentrations had different fumigant effects on *R. dominica* larvae. The mortality (determined from the number of adults obtained) strongly increased with the increased *L. nobilis* oil concentration but somewhat less with the other two EOs. Differences between the efficacy of *L. nobilis* and other EOs were evident from the 50 µl/L. LC_{50} estimates were 2.1, 7.0 and 7.4 (\log_{10} µl/L + 0.1) for *L. nobilis*, *L. intermedia* and *M. piperita*, respectively (Table 2). The back-transformed *L. nobilis* LC_{50} was approximately 128 µl/L and 5 orders of magnitude more effective than the other essential oil. The fumigant tests on larvae gave the highest mortality (100%) at 200 µl/L *L. nobilis* essential oil (Fig. 2a).

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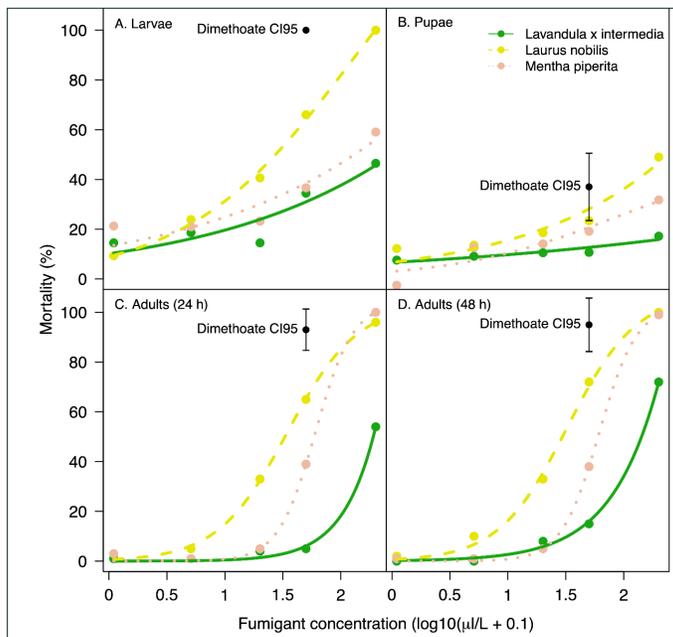


Figure 2. Essential oil dose response on mortality of *Rhizopertha dominica* (data were fitted to a three-parameter log-logistic model). The mean mortality and 95% confidence interval for dimethoate is plotted at the concentration applied as a comparator.

Table 2. Dose–response parameters of the log-logistic model for essential oils on *Rhizopertha dominica*.

Treatments	Upper limit (d)	Curve slope (b)	LC ₅₀
Larval stage			
<i>Lavandula x intermedia</i>	1072.103 (5419.319)*	-0.669 (0.185)	6.956 (8.572)
<i>Laurus nobilis</i>	176.954 (88.372)	-1.389 (0.423)	2.108 (0.738)
<i>Mentha piperita</i>	1490.234 (5333.455)	-0.640 (0.142)	7.365 (6.275)
Pupal stage			
<i>Lavandula x intermedia</i>	151.611 (1154.299)	-0.405 (0.456)	7.599 (24.709)
<i>Laurus nobilis</i>	2944.843 (24036.059)	-0.852 (0.228)	7.150 (10.119)
<i>Mentha piperita</i>	49.836 (62.979)	-1.422 (1.199)	1.935 (1.900)
Adult stage (24 h exposure)			
<i>Lavandula x intermedia</i>	1761.8 (18497.0)	-3.672 (1.763)	3.242 (3.112)
<i>Laurus nobilis</i>	103.60 (11.999)	-3.315 (0.965)	1.539 (0.108)
<i>Mentha piperita</i>	104.00 (11.600)	-6.203 (3.466)	1.782 (0.0899)
Adult stage (48 h exposure)			
<i>Lavandula x intermedia</i>	1423.9 (15582.0)	-2.563 (0.863)	3.446 (4.804)
<i>Laurus nobilis</i>	107.63 (10.810)	-3.367 (0.965)	1.513 (0.909)
<i>Mentha piperita</i>	103.32 (11.625)	-6.108 (3.282)	1.788 (0.092)

* Standard errors are in parentheses.

Dose-response parameters for *R. dominica* pupae after 48-h exposure to the EOs in Table 2. In contrast to the results for the larvae, the mortality caused by all EOs was low and adult emergence was not strongly suppressed, even at the highest concentrations. Pupal mortality was the lowest with *L. intermedia* essential oil. The

pupal mortality (based on the number of emerged adults) increases gradually with increasing concentration of *M. piperita* and *L. nobilis* oils. However, these EOs on *R. dominica* pupae gave well under 50% mortality. Only *L. nobilis* oil gave mortality close to 50% at the highest concentration, a result similar to dimethoate (Fig. 2b).

Dose-response parameters for *R. dominica* adults after 24-h exposure to different essential oil doses are shown in Table 2. LC₅₀ estimates were 1.5, 1.8 and 3.2 (log₁₀ µl/L +0.1) for *L. nobilis*, *M. piperita* and *L. intermedia*, respectively. Back-transformed estimates for *L. nobilis* and *M. piperita* estimates were similar at 35 and 60 µl/L, respectively. Both EOs there were more efficacious than that of *L. intermedia*. The mortality at two lowest concentration (1 and 5 µl/L) was negligible. However, the differences between the three oils changed with the concentration. The oil from *L. nobilis* was clearly better at 50 µl/L, but by 200 µl/L *M. piperita* oil was equally effective.

The comparison of the mortality rates after 24 and 48 h of exposure to plant EOs revealed similar curves based on the exposure time. The three model parameters were similar between the two assessment times (Table 2) and consequently the fitted curves were quite similar (Fig. 2c, d). The extra 24 h of exposure provided minimal additional benefit.

DISCUSSION

The insecticidal (fumigant) effects measured for the EOs from studies plants (mint, lavender, laurel) indicated they have potential for control of *R. dominica*. They had significant effects on the mortality of *R. dominica* at various developmental stages (larva, pupa and adult). The fumigant activity tests on larvae gave complete mortality with 200 µl/L *L. nobilis* oil. Similar results were obtained with adults. Complete mortality was obtained at high concentrations of *L. nobilis* and *M. piperita* oils. However, the effects of the EOs were generally quite low on the pupal stage. The fumigant effect tests on pupae had the highest effect with *L. nobilis* oil (49% mortality), followed by *M. piperita* and *L. intermedia* EOs. It is reported that the efficacy of fumigants can be low for pupae, as pupal respiration rate is especially low in insects found in grain kernels (Hagstrum, Phillips, & Cuperus, 2012). Similarly, the efficacies of various EOs and chemicals have been investigated in *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) (Güdek & Çetin, 2016), *Acanthoscelides obtectus* Say, 1831 (Coleoptera: Bruchidae) (Papachristos & Stamopoulos, 2002), *Ephestia cautella* (Walker, 1863) (Lepidoptera: Pyralidae), *O. surinamensis* and *T. castaneum* (Isikber et al., 2004) control, and pupae were generally more resistant. In the present study, similarly, the essential oil activities in the larval and adult stages were higher than the pupal stages at all concentrations.

Across all the developmental stages, *L. nobilis* oil gave the highest activity and *L. intermedia* the lowest.

The mortality from 50 µl/L *L. intermedia* oil was 15% in adults stage 48 h whereas it was 72% at the highest dose (200 µl/L). Similar to these findings, Eli Shaaya et al. (1991) reported that 15 µl/L lavender essential oil had fumigant effects on *R. dominica*

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adults with complete mortality. Rozman, Kalinovic, & Korunic (2007) stated that the fumigant effect of lavender essential oil was high on *R. dominica*, and the linalool and camphor in the lavender essential oil led to complete mortality within 24 h at the lowest concentration (0.1 µl/720 ml). In another study, Ebadollahi, Safaralizadeh, & Pourmirza (2010) reported that the fumigant effect of *Lavandula stoechas* L., 1753 (Lamiales: Lamiaceae) essential oil on *R. dominica* after 24 and 48 h with LC₉₅ being 80.6 and 28.9 µl/L, respectively. Main constituents reported were 1,8-cineole (7.02%), γ-cadinene (5.33%), t-cadinol (5.07%), p-mentha-1-en-8-ol (5.02%) and caryophyllene (5.01). In the present study, however, *L. intermedia* essential oil containing menthone (53.5%), menthol (17.2%) and menthofuran (5.4%) did not produce complete mortality of *R. dominica* adults. It is concluded that this lower effect might be due to the variations in the chemical compositions of the essential oil even though coming from plants in the same genera.

Mentha piperita essential oil LC₅₀ was almost 60 µl/L in adults after 24 h of, and the mortality at 200 µl/L was complete. Similar to these findings, Tripathi, Veena, Aggarwal, & Sushil (2000) reported that *M. piperita* essential was lethal to *T. castaneum* and *C. maculatus*. Mackled, El-Hefny, Bin-Jumah, Wahba, & Allam (2019) demonstrated that 70 µl/L *M. piperita* essential oil, the main components of which included menthol (33%) and menthone (20%), had a fumigant effect on *R. dominica* after 72 h giving complete mortality. In the present study, it was found that the fumigant effect of 200 µl/L *M. piperita* oil was 100% in the shorter time (24 h). In another study, Çam, Karakoç, Gökçe, Telci, & Demirtaş, (2012) reported that 10 µl/L *M. piperita* essential oil had fumigant effects on *S. granarius* with mortality of 58% after 24 h. Khani et al. (2017) reported 95% mortality in *S. oryzae* adults after exposure to 299 µl/L *M. piperita* essential oil. In the present study, 200 µl/L *M. piperita* essential oil gave complete mortality in *R. dominica* adults. Although the pest species tested were different, the effects were similar. Akkuş, Gözüaçık, & Gültekin (2021) investigated the fumigant toxicity of *Mentha longifolia* L., 1759 (Lamiales: Lamiaceae) essential oil on *R. dominica* adults with 10 µl/petri dish giving complete mortality after 24 h. It is suggested that the lower response seen in the current study was due to the variations between the chemical compositions of the plant essential oil.

In the present study, the mortality caused by 35 µl/L *L. nobilis* essential oil was about 50% in adults but at 200 µl/L was mortality was complete in both larval and adult stages of *R. dominica*. Similarly, Mediouni Ben Jemâa et al. (2012) reported that the essential oil of *L. nobilis* from Morocco, Algeria, and Tunisia was lethal to *R. dominica* with LC₅₀ of 67.9, 99.0 and 113 µl/L, respectively. Major components also reported as 1,8 cineole 38.86, 34.62 and 24.55% and Linalool 9.45, 12.57 and 17.67% for the essential oils obtained in these countries. Kırpık, Kılıçlı, & Yıldız Asker (2019) reported that 100 µl/L *L. nobilis* essential oil gave complete mortality of *R. dominica* after 24 h. In the present study, the mortality with *L. nobilis* essential oil with high 1,8-cineole content (58%) was 96% at the highest concentration (200 µl/L) after 24 h. It is suggested that the different chemical compositions of plant EOs are likely to lead to different findings.

Data from current experiments indicate that all the EOs of all three plants have fumigant effects on *R. dominica* at promising levels, especially the oils of *L. nobilis* and *M. piperita*. The EOs of both plants could be employed as a substitute instrument to chemical insecticides in pest control.

Despite the high effects obtained with the administration of EOs in larval and adult stages, the same effect was not observed on the pupal stage. Thus, the developmental stage of the pest should not be ignored in the recommendations about treating infested products.

Finally, further studies should be conducted on the developmental stages inside the kernel, i.e. the larval and pupal stages. It is recommended that future studies also be conducted to test the penetration of the essential oils into large quantities of the product and to prepare new formulations to improve insecticidal duration and efficacy.

ACKNOWLEDGEMENTS

This study is a part of the master degree thesis of the first author and was sponsored by the Scientific Research Fund of Malatya Turgut Özal University (Grant Project No: BAP-YL3). We thank Batı Akdeniz Agricultural Research Institute (Turkey) for supplying the *L. intermedia* and *L. nobilis* essential oils and analyzing all oils. We are also grateful to Prof. Dr. Ian T. Riley for his assistance during the statistical analysis and manuscript preparation.

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***Crematogaster gadagkari* sp. nov. (Hymenoptera: Formicidae), a New Species of an Acrobat Ant from India**

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ABSTRACT

We describe a new species *Crematogaster gadagkari* sp. nov. based on worker specimens from the North East Himalaya, India collected in 2019. It resembles *C. rogenhoferi* Mayr, 1879 and *C. himalayana* Forel, 1902, but can be distinguished from both species on the basis of sculpture and propodeal spine length. Also updated an identification key to the Indian *Crematogaster* species. In addition, a DNA barcode for the newly described species is also provided with Genbank accession number "OP807010".

Keywords: Myrmicinae, morphology, DNA barcoding, key, taxonomy.

INTRODUCTION

Crematogaster Lund, 1831 is a diverse genus of ants that is distributed worldwide. It is represented by 522 valid species and 260 subspecies from all over the world (Bolton, 2023). *Crematogaster* ants exhibit a wide range of ecological adaptations, living in habitats ranging from tropical rainforests to deserts (Hölldobler & Wilson, 1990). They are also known for their diverse behaviors, such as tending to honeydew-producing insects, building nests in a variety of substrates, and engaging in territorial defense (Madden & Young, 1992; Longino, 2003). These ants commonly found in the forest areas and prefer to live in shrubby habitats. Mostly *Crematogaster* species build arboreal nests but some species belonging to the subgenus *Orthocrema* nests in ground (Hosoishi, Yamane, & Ogata, 2010; Blaimer, 2012a, b; Palmer & Brody, 2013). Phylogenetic analysis has classified the genus into two subgenera *Crematogaster* and *Orthocrema* (Blaimer, 2012c, d). Recent advances in molecular techniques and morphological analysis have provided new insights into the evolutionary history and relationships of these ants, leading to a renewed interest in their taxonomy (Hosoishi et al, 2023)

The taxonomy of *Crematogaster* ants has been a topic of debate and revision for many years. While the genus was originally established by Lund, P.W. in 1831 on the basis of type species *Crematogaster scutellaris* (Olivier, 1792) and subsequent designation by Bingham, in 1903 and has undergone several revisions since then, with new species being added and old ones being reclassified. However, some taxonomic contributions to the genus include; Buren (1959, 1968) from North America; Longino (2003) from Costa Rica; Blaimer (2010, 2012a, b) from Madagascar; Hosoishi & Ogata (2008, 2009, 2015, 2017) from South East Asia; Fernández & Serna (2019), Pedraza & Fernández (2019) from Colombia; Sharaf, Aldawood, & Hita Garcia, 2019, Sharaf & Aldawood (2022) from Arabia.

From India, the genus is currently represented by valid 34 species and subspecies (Akbar, Bharti, & Wachkoo, 2023). Despite previous research on the diversity and distribution of ants in India, the taxonomy of the *Crematogaster* genus is an area of ongoing research, with much still to be learned about their classification. During the present study, we describe a new species *Crematogaster gadagkari* sp. nov. from India. This study also updates the identification key to the known species of the genus, providing a valuable resource for future research in this field. In addition, a DNA barcode for the newly described species is provided with GenBank accession number "OP807010". These findings contribute to the ongoing research on the taxonomy of the *Crematogaster* genus in India and demonstrate the importance of continued exploration and discovery in this area.

MATERIALS AND METHODS

Taxonomic analysis was conducted using a Nikon SMZ 1500 stereo zoom microscope with a maximum magnification of 112.5 x. Digital images of the specimens were prepared using a Nikon SMZ 1500 stereomicroscope fitted with an MP (Micro

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Publisher) digital camera and Auto Montage (syncroscopy, a division of Synoptics Ltd.) software. All the images were cleaned with Adobe Photoshop CS5 and Helicon Filter 5. Morphological measurements were recorded in millimetres with an oculo micrometer fitted on a Nikon SMZ 1500 stereomicroscope. Additional images (CASENT0914078 and CASENT0908582) were provided by <http://www.antweb.org/>. Morphological terminology and standard measurements follow Hosoiishi (2020).

Head Width (HW): Maximum width of head in full-face view, excluding the eyes.

Head Length (HL): Perpendicular distance from vertex margin to line tangent anterior most projections of clypeus in full-face view.

Scape Length (SL): Length of the first antennal segment, excluding the neck and basal condyle.

Eye Length (EL): Maximum length of the compound eye. Pronotal Width (PW): Maximum width of the pronotum in dorsal view.

Weber's Length of the mesosoma (WL): Diagonal length, measured in lateral view from the anterior margin of the pronotum (excluding the collar) to the posterior extremity of the propodeal lobe.

Propodeal Spine Length (PSL): Measured from tip of propodeal spine to closest point on outer rim of propodeal spiracle.

Petiole Length (PtL): Length of the petiole in lateral view.

Petiole Width (PtW): Maximum width of petiole in dorsal view. Petiole Height (PtH): Height of the petiole in lateral view.

Petiole Height (PtH): Height of the petiole in lateral view

Postpetiole Length (PpL): Length of the postpetiole in lateral view.

Postpetiole Width (PpW): Maximum width of postpetiole in dorsal view, excluding the helcium.

Indices

Scape Index (SI): $SL/HW \times 100$

Cephalic Index (CI): $HW/HL \times 100$.

Eye Index (EI): $EL/HW \times 100$.

Petiole Height Index (PtHI): $PtH/PtL \times 100$.

Petiole Width Index (PtWI): $PtW/PtL \times 100$.

Postpetiole Width Index (PpWI): $PpW/PpL \times 100$.

Waist Index (WI): $PpW/PtW \times 100$

Molecular Analysis: DNA was extracted from whole ant specimens using QiagenDNeasy® kit, following the manufacturer's protocols. Rest of the respective individuals were kept as voucher specimens at PUPAC. Mitochondrial cytochrome oxidase 1 (COI) gene barcoding region (658 bp) near the 5' terminus of the *cox1* gene was amplified by following standard protocol (Hebert et al, 2003). Standard primers used were: forward primer (LCO 1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'),

and reverse primer (HCO 2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR reactions were carried out in 96-well plates. Thermo cycling consisted of an initial denaturation of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. PCR was performed using a C1000™ Thermal Cycler. The amplified product was sent to Medauxin Pvt. Ltd., India. DNA sequence obtained was checked for homology, insertions and deletions, stop codons, and frame shifts by using NCBI BLAST.

Depositories: Specimens were deposited in PUAC “Punjabi University Patiala Ant Collection” at Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India.

RESULTS

COX I sequence was submitted to GenBank and the Barcode of Life Database (BOLD, <http://www.boldsystems.org>) and available publically with accession number “OP807010.”

Family: Formicidae Latreille, 1809

Subfamily: Myrmicinae Lepeletier de Saint-Fargeau, 1835

Genus: *Crematogaster* Lund, 1831

Type-species: *Formica scutellaris* (obsolete combination of *Crematogaster scutellaris*), by subsequent designation of Bingham, 1903: 124.

Crematogaster gadagkari sp. nov. (Fig. 2)

Type Material. Holotype (worker): India: Sikkim: Namchi, 1300 m, 27.16° N, 88.36°E, handpicking, 09.xi.2019 [PUAC-T 501]. Paratypes: 10 (w.) (PUAC- T 502-T 512), same data as holotype; 7 (w.), Sikkim: Dentam, 1500 m, 27.25° N, 88.13° E, 15.xi.19, hand picking method, leg. Tarun Dhadwal [PUAC T 515-T 522] (Fig. 1).

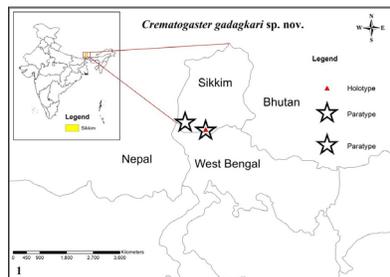


Figure 1. A map of India showing locations of Holotype and Paratypes of *Crematogaster gadagkari* sp. nov.

Measurements: (Worker mm, n = 10; given are ranges; holotype measurements in parentheses):

HW 0.99-1.02 (1.01mm); HL 0.96-1.05 (1.05 mm); SL 0.66-0.72 (0.70 mm); EL 0.48-0.51 (0.49 mm); WL 1.11-1.14 (1.12 mm); PSL 0.23-0.25 (0.25 mm); PtL 0.39-0.42 (0.40 mm); PtH 0.20-0.23 (0.23 mm); PtW 0.33-0.36 (0.36 mm); PpL 0.26-0.30 (0.30

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mm); PpW 0.30-0.33 (0.33 mm); CI 97.14-103.12 (96.19 mm); SI 66.66-70.58 (66.66); PtHI 51.28-54.76 (57.50); PtWI 84.61-85.71 (90); PpWI 110.00-115.38 (110); WI 90.90-91.66 (91.66); EI 48-50 (48.51) .

Diagnosis: Head coarsely, very finely longitudinally striate and mesonotum reticulated. Mesopleuron and lateral sides of the propodeum reticulated, petiole and postpetiole smooth, Propodeal spine long and straight, petiole is longer than broad with lateral margins weakly convex; postpetiole bilobed with a longitudinal median sulcus in dorsal view; subpetiolar process developed forming a spine directed anterior-ventrally. Head, mesosoma and gaster are black and shiny; Legs and scape brownish red. However few specimens are completely Jet black and shiny. Body generally without any erect or suberect hair.

Description: Head in full face view, as long as broad with almost straight posterior margin and rounded occipital corners, lateral sides are convex; frontal carinae short not extending up to midlength of head; mandible with five teeth; Anterior clypeal margin feebly convex except medially; eyes small (EI 48-50), projecting slightly beyond the mid length of the head; antennae 12-segmented, with a club of an apical 3-larger segment, scape short (SI 66.66-70.58) reaching almost up to the posterior corners of the head.

In lateral view, pronotum higher than metanotum; meso-metanotal groove distinct forming a deep concavity; metanotum with distinct ridges; propodeal spine long, pointed diverging posterolaterally; propodeal spiracles dorsoventrally oval in shape. In dorsal view, petiole is longer than broad with lateral margins weakly convex; postpetiole bilobed with a longitudinal median sulcus in dorsal view; subpetiolar process developed forming a spine directed anterior-ventrally.

Sculpture: Head coarsely, very finely longitudinally striate. Mandibles and clypeus longitudinally striate; pronotum reticulate and striated at sides, mesonotum reticulated, metanotum longitudinally striate; mesopleuron and lateral sides of the propodeum reticulated, petiole and postpetiole smooth, dorsal gastral tergites smooth.

Pilosity and pubescence: Body sparsely pilose, short suberect hair present over the head; scape with abundant suberect to decumbent setae; clypeus with suberect setae, anterior clypeal margin with two to three pairs of longer setae, mixed with some shorter setae on the sides.

Head, mesosoma and gaster are black; Legs and scape brownish red. However few specimens are completely black.



Figure 2. *Crematogaster gadagkari* sp. nov. a) head in full face view, b) body in lateral view, c) body in dorsal view.

Remarks: The species is closely related to the *C. rogenhoferi* Mayr, 1879 (Figs. 3a, b, 4a) and *C. himalayana* Forel, 1902 (Figs. 3c, d, 4b). having head entirely sculptured not smooth, pronotum reticulated and propodeal spine is distinctly longer than metanotum.

From *C. rogenhoferi* Mayr, 1879, it can be distinguished by following characteristics; pronotum convex, metanotum forming a distinct ridges (pronotum flat, metanotum depressed in *C. rogenhoferi*); petiole is longer than broad with lateral margins weakly convex (petiole as broad as long with sides angular in the middle in *C. rogenhoferi*); propodeal spine long and straight in dorsal view (propodeal spine short and curved inward in dorsal view in *C. rogenhoferi*); in dorsal view postpetiole is not bulbous and enlarged (in dorsal view postpetiole is bulbous/enlarged in *C. rogenhoferi*) head and mesosoma longitudinally striate (head and mesosoma longitudinally rugulose in *C. rogenhoferi*); Head and mesosoma and gaster black and shiny (Head, mesosoma and gaster reddish brown to dark brown and opaque in *C. rogenhoferi*)

From *C. himalayana* Forel, 1902, it can be distinguished by the following characteristics; head coarsely, very finely longitudinally striate (head irregularly striated and reticulated in *C. himalayana*); pronotum reticulate (pronotum coarsely rugulose at sides in *C. himalayana*); petiole node weakly convex at margins (petiole node rounded in *C. himalayana*); petiole and postpetiole smooth and shiny (petiole and postpetiole reticulated in *C. himalayana*), propodeal spines straight and do not arc outwards (propodeal spine arc outwards in *C. himalayana*), Head and mesosoma and gaster black and shiny (Head, mesosoma ferruginous red and gaster somewhat black and opaque in *C. himalayana*).

Etymology: The species is named in honour of “Prof. Raghavendra Gadagkar” a distinguished Evolutionary biologist based at Centre for Ecological Sciences, Indian Institute of Science in Bangalore, India.

Ecological and biological notes: The workers were handpicked from a tree trunk and ground in Namachi, a village located in the Indian state of Sikkim. Namachi is situated at an average elevation of 1600 meters above sea level, and despite being located in a tropical region, it experiences a relatively cooler climate than other areas in the region. The average daily temperature in Namachi is around 28 degrees Celsius. The village is surrounded by dense forest areas, which provide an excellent habitat for ants and other insects. These forests also contribute to the region’s biodiversity and provide a natural source of beauty.

Identification key to the known species of genus *Crematogaster* Lund, 1831 from India.

The identification key to the known species of genus *Crematogaster* from India was recently provided by Akbar et al (2023). The new species runs to couplet 14 and the key is modified as follows.

14a. Pronotum reticulate; propodeal spines directed downward.....14b
 Pronotum longitudinally striate; propodeal spines upcurved*C. flava* Forel, 1886

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14b. Petiole as long as broad, sides angular in the middle in dorsal view; propodeal spine curved posteriorly in dorsal view*C. rogenhoferi* Mayr, 1879

Petiole longer than broad, sides weakly convex in dorsal view; propodeal spines straight, directed posterolaterally in dorsal view.....*C. gadagkari* sp. nov.

[**Note:** From couplet 15 onwards there are no changes to the key presented by Akbar et al (2023) and we refer to that publication].

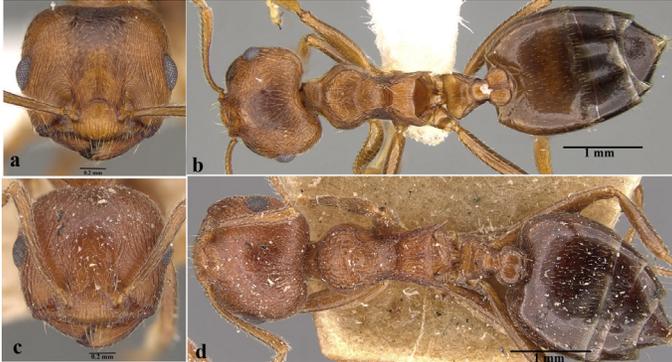


Figure 3. Head in full face view and body in dorsal view, a, b) *C. rogenhoferi* Mayr, 1879 (AntWeb-CASENT0914078, Photographer- Z. Lieberman), c, d) *C. himalayana* Forel, 1902 (AntWeb-CASENT0908582, Photographer- Will Ericson).



Figure 4. Body in profile view, a) *C. rogenhoferi* Mayr, 1879 (From AntWeb- CASENT0914078, Photographer- Z. Lieberman), b) *C. himalayana* Forel, 1902 (from AntWeb- CASENT0908582, Photographer- Will Ericson).

ACKNOWLEDGMENTS

Financial assistance rendered by the Department of Science and Technology/ Science and Engineering Research Board (SERB), (Project File No. EMR/2017/000660), Govt. of India, New Delhi is gratefully acknowledged. We also thank Forest and Wildlife

Department, Govt. of Sikkim for granting the permission to collect the research material vide Order No. 78/GOS/FEWMD/BDR/PCCF/Secy/R&E/36 dated 11.09.2019.

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First Confirmed Locality Record of Ladybird Beetle *Synona melanopepla* (Mulsant, 1850) (Coleoptera: Coccinellidae) from Nepal

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ABSTRACT

Synona melanopepla (Mulsant, 1850) is a ladybird beetle belonging to the subfamily Coccinellinae of the family Coccinellidae. This species has been confirmed for the first time from Nepal through this study. A single specimen of this species was collected from Ranibari Community Forest, Kathmandu, Nepal.

Keywords: First record, Beetle, Coccinellidae, Ranibari Community Forest, Kathmandu

INTRODUCTION

All ladybird beetles belong to the family Coccinellidae of Order Coleoptera. This family is one of the most diversified beetle groups which comprises more than 375 genera (Nedvěd, 2015; Soares et al., 2021) and 6000 species (Vandenberg, 2002). Genus *Synona* is also a coccinellid beetle that consists of five species *S. philippinensis*, *S. melanaria*, *S. melanopepla*, *S. obscura*, and *S. consanguinea* from Oriental and Australasian regions (Poorani, Ślipiński, & Booth, 2008).

The genus *Synona* is considered as a potential predator that usually prey on nymphs of plataspid bug, specifically *Coptosoma ostensum* (Subramanyam, 1925), tessarotomid bug specifically *Cumare pallida*, (Afroze & Uddin, 1998; Monteith, 2006), psyllids, pentatomids (Poorani, 2008) and aphids (Poorani, 2023). It was synonymized with *Synona rougeti* (Mulsant, 1866), *Lemnia melanopectera* lablokoff-Khnzorian, 1978, and *Lemnia (Synia) martini* lablokoff-Khnzorian, 1984 (Poorani et al., 2008). *Synona melanopepla* (Mulsant, 1850) was originally described from the East Indies as *Synia melanopepla*. The species was found previously distributed only in five different countries; India (Mulsant, 1850), Vietnam (Poorani et al., 2008), and Bhutan (Dorji, Loday, & Vorst, 2019), Sri Lanka, and China (Poorani, 2023). Poorani (2023) enlists Nepal as a distributional range of the species but without any confirmed locality in the country.

MATERIALS AND METHODS

Study Area and Sampling

As a part of the biodiversity survey in the remnant forest patches of Kathmandu valley, study was conducted in the Ranibari Community Forest (RCF) Kathmandu. The forest lies at an altitude of 1330 m asl and geographically stands at 27° 43' 50.94" N latitude and 85° 19' 15.77" E longitude (Fig. 1) which comprises a total area of 9.5 hectares. The forest is dominated by mixed vegetation like Bamboo, *Castano-opsis indica*, *Ficus lacor*, *Pinus roxburghii*, *Melia azedarach*, *Quercus glauca*, *Ziziphus incurva*, *Engelhardia spicata*, and *Schima wallichii*. The survey was conducted by establishing three systematic plots each of 10m X 10m as a lattice grid within 200 m intervals between two consecutive plots in the forest. During the survey periods, three sampling techniques were extensively used that includes beating (McCravy, 2018), Handpicking (Gobbi et al., 2018) and Pitfall traps (Pompeo, Oliveira Filho, Klauberg Filho, Mafra, & Baretta, 2020).

Collection, Preservation and Identification:

The survey was conducted in October 2021. The species was collected following hand-picking method and immediately transferred into the killing jar containing ethyl acetate soaked with a cotton cloth. The collected specimen was taken in the Entomological Laboratory of the Central Department of Zoology, Tribhuvan University for the detailed taxonomic study. The specimen was carefully studied under the

*First Confirmed Locality Record of Ladybird Beetle *Synona melanopepla**

stereo-microscope model BEL-3020b (10X4.5). The confirmation of the species was made following identification features by using Poorani et al. (2008). After completing identification, the specimen of the species was photographed (Dorsal and Ventral View), and safely preserved in the Central Department Zoology Museum of Tribhuvan University, Kathmandu, Nepal with systematic labelling (catalogue number: CDZMTU-COL20).

RESULT

A single notable individual of the species of beetle from the study site. The species was confirmed as *Synona melanopepla* which was the first confirmed locality record for Nepal. This species was encountered only once, from an undisturbed second plot through out the survey (Fig. 1).

***Synona melanopepla* (Mulsant, 1850)**

Body size: 7.18 mm in length and 6 mm in width (Fig. 2). Body circular and hemispherical. Head dark orange. Anterior clypeal border semicircular. Pronotum dark yellow-orange with median black marking. Scutellum and elytra black and glabrous. Antenna yellowish brown with the last antennomere darker and wedge shaped. Pronotal punctures large and dense. Punctures in elytra finer and widely placed as compared to pronotal punctures. Elytra has several coarse and deeply pitted punctures on an anterolateral areas which is distinguishing morphological character from similar looking *S. melanaria* in which coarse punctures are not present in anterolateral areas. Ventral side brownish with black elytral epipleura. Prosternal intercoxal process with subparallel carina reaching up to middle of prosternum. The sexes of the species was not separated.

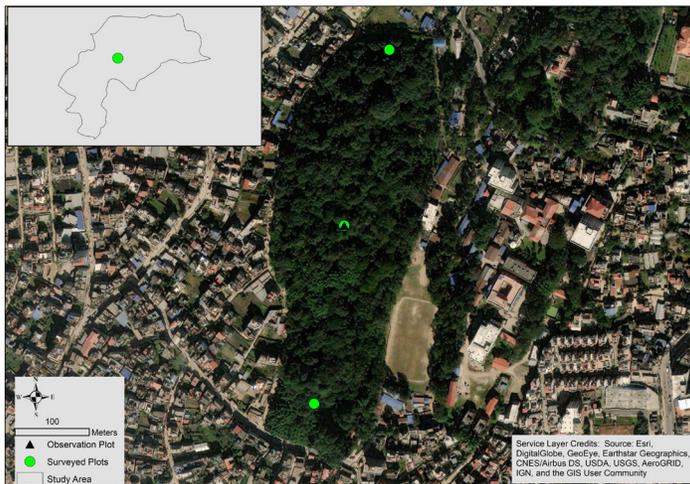


Figure 1. Map showing study plots with beetle observation plot.



Figure 2. *Synona melanopepla* (Mulsant). a) dorsal view, b) ventral view (scale bar- 1mm).

DISCUSSION

This species is commonly distributed in neighbouring India and other adjacent countries. Nepal is located near Assam, Bihar, and Uttar Pradesh in northern India, where *S. melanopepla* is found. This study highlights the current new habitat and range extension of the species. This species feeds on pests which are also known to occur in Nepal viz. *Coptosoma* (Westwood, 1837), *Megacocta cribraria* (CABI, 2022), psyllids, pentatomids, and aphids. The presence of *Ficus* sp. plant in RCF may also support the existence of this species which was documented preying psyllids on *Ficus* sp. by Poorani (2008). This shows the possibility of this species being native to Nepal.

ACKNOWLEDGEMENTS

The authors are very thankful to the Department of Forest and Soil Conservation, Babarmahal, Kathmandu for granting the research and collection permission to the project and Central Department of Zoology for allowing us to use the laboratory. We would also like to thank Dr. Janakiraman Poorani for guidance and confirmation of species.

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New Record of Predatory Thrips *Aeolothrips wittmeri* Priesner (Thysanoptera, Aeolothripidae) from Türkiye

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ABSTRACT

Up to the present, 16 species of the genus *Aeolothrips* have been recorded from Türkiye. This study identified a new predatory species, *Aeolothrips wittmeri* Priesner from cucumber fields in Diyarbakır Province, Türkiye. The species is similar to another predatory thrips *Aeolothrips gloriosus* Bagnall in color of last abdominal segments in which abdominal segments VIII–X and sometimes VII are dark brown and other parts of the body are yellow to light brown with pale brown spots. The characters separating these two species are provided in this manuscript with illustrations.

Keywords: cucumber fields, morphological description, checklist, Diyarbakır.

Büyük, A.H., Mutlu, Ç., & Mirab-Balou, M. (2024). New record of predatory thrips *Aeolothrips wittmeri* Priesner (Thysanoptera, Aeolothripidae) from Türkiye. *Journal of the Entomological Research Society*, 26(1), 33-37.

Received: May 17, 2023

Accepted: November 22, 2023

INTRODUCTION

The family Aeolothripidae is the third largest family in the order Thysanoptera, after Phlaeothripidae and Thripidae with 220 extant species in 23 genera worldwide (Alavi & Minaei, 2018; Mirab-balou, 2019). Members of this family are facultative predators of other small arthropods, and feed on both floral tissues as well as on thrips and mites that live in flowers. However, considerable number of species are obligate predators in warmer parts of the world, (Tyagi et al., 2008). More than 50% of the described aeolothripid species are placed in the genus *Aeolothrips* Haliday (ThripsWiki, 2023).

Aeolothrips genus is essentially Holarctic, being distributed in the Palaearctic as well as the Nearctic regions, and regarded as the most species-rich in the family Aeolothripidae with 112 species around the world (ThripsWiki, 2023). So far, 16 species of *Aeolothrips* have been recorded from Türkiye (Tunç & Hastenpflug-Vesmanis, 2016; Uzun Yigit, Demirozer, & Minaei, 2022). This study reports another predatory *Aeolothrips* species, *A. wittmeri* Priesner in Türkiye based on two females collected from cucumber fields in Diyarbakır province. It is compared with related species with illustrations.

MATERIALS AND METHODS

Specimens were collected by beating cucumber [*Cucumis sativus* (Cucurbitaceae)] leaves and flowers onto a plastic tray, from Diyarbakır province, Türkiye. The specimens were removed with a fine brush into a collecting vial containing 70 % ethyl alcohol. They were then mounted onto slides according to the method described by Remani, Thippeswamy, Ramasamy, & Shivalingegowda (2023). Slides were examined using a Nikon Eclipse 80i microscope, and micro pictures were acquired using a Nikon DS-Vi1 camera placed at the top of the microscope. Specimens are deposited in the collection of Department of Plant Protection, College of Agriculture, Ilam University, Iran (ILAMU). The species was identified according to appropriate identification keys (Alavi & Minaei, 2018; Mirab-balou, 2019).

RESULTS

The species discussed below belongs to the genus *Aeolothrips*, family Aeolothripidae and can be recognized by the following characters: bicolored body, antennae 9-segmented, III and IV with linear sensorium; head without long setae behind the compound eyes, pronotum without prominent posteroangular setae and the forewings broad with the apex rounded and usually with two dark transverse bands, posterior margin of forewing is dark except at the base and apex.

***Aeolothrips wittmeri* Priesner**

Aeolothrips wittmeri Priesner, 1935: 315 (Type species, female at Senckenberg Museum, Frankfurt).

New Record of Predatory Thrips Aeolothrips wittmeri

Material examined: TÜRKİYE, Diyarbakır, 2♀♀, on flowers of cucumber, *Cucumis sativus* (Cucurbitaceae), 17.7.2022, Leg. A.H. Büyük.

Morphological description: Female macroptera (Fig. 1a); body bicolored, head and thorax yellow with variable brown areas medially, pronotum with a brownish median longitudinal stripe (Fig. 1c), abdominal segments I–IV largely yellow, V–VI mostly brown, VII–X dark brown; antennal segments brown except III yellow at basal third (Fig. 1b); legs yellow, mid and hind tibiae dark brown, mid- and hind femora with brown area apically (Fig. 1a); forewings with two dark transverse bands, ring vein pale around wing apex but weakly shaded between the dark bands, posterior margin of forewing is dark except at the base and apex (Fig. 1d).

Antennae 9-segmented, segment III with linear sensorium about 0.5 of segment length, IV with sensorium broader and longer, curving around segment apex. Head and pronotum with no long setae; mouth cone long, maxillary palps 3-segmented. Abdominal tergite I with weak transverse reticulation; sternite VII with lateral two pairs of marginal setae arising sub-marginally, two pairs of accessory setae arising close to margin, between marginal setae S1 and S2.



Figure 1. *Aeolothrips wittmeri*, a) female, b) antenna (right), c) head and pronotum, d) fore wing, showing dark posterior margin.

Distribution: Egypt, Iran (Alavi, Mosallaei, & Sajjadi, 2012; Minaei 2013)

Checklist of *Aeolothrips* species previously recorded from Türkiye

Aeolothrips albicinctus Haliday

Aeolothrips astutus Priesner

Aeolothrips balati Pelikan

Aeolothrips collaris Priesner

Aeolothrips cursor Priesner
Aeolothrips ericae Bagnall
Aeolothrips fasciatus (Linnaeus)
Aeolothrips gloriosus Bagnall
Aeolothrips heinzi zur Strassen
Aeolothrips intermedius Bagnall
Aeolothrips linarius Priesner
Aeolothrips melaleucus Haliday
Aeolothrips priesneri Knechtel
Aeolothrips propinquus Bagnall
Aeolothrips tenuicornis Bagnall
Aeolothrips versicolor Uzel

DISCUSSION

Among *Aeolothrips* species from Türkiye, except micropterous *A. cursor* and the micropterous form of *A. albicinctus*, posterior margin of forewing is pale medially between two dark cross bands whereas in four species, *A. gloriosus*, *A. melaleucus* and *A. versicolor* and *A. wittmeri* the posterior margin of forewing is dark except base and apex. However, *A. gloriosus* and *A. wittmeri* are distinguished from *A. melaleucus* and *A. versicolor* by the narrow form of the band along the forewing posterior margin between the two cross bands, in contrast to the other two species that have a wider band (Minaei, 2014; Alavi & Minaei, 2018). The species have been reported from Iran (Mirab-balou, 2018).

The females of *A. wittmeri* are readily differentiated from *A. gloriosus* by the following characters: antennal segments I and II brown (Fig. 1b) (vs. yellow in *gloriosus*); mid and hind tibiae brown (Fig. 1a) (vs. yellow in *gloriosus*); pronotum with a brownish median longitudinal stripe (Fig. 1c) in contrast to *gloriosus* without brownish median longitudinal stripe. In male of *A. wittmeri*, abdominal tergite IX has dark spine-like seta on either side of posterior margin, but *A. gloriosus* with no dark spine-like setae (Alavi & Minaei, 2018). In conclusion, accurately identification of *A. wittmeri* from various geographical locations is of paramount importance for taxonomy, conservation, agriculture, and molecular research. Accurate species identification is crucial to enhance our understanding of its distribution, ecological characteristics, and evolutionary trajectory. The results of the current study will facilitate the development of efficient management strategies against *A. wittmeri*.

ACKNOWLEDGEMENTS

This paper is extracted from part of the M.Sc. thesis of the senior author, who was financially supported by Harran University, Şanlıurfa, Türkiye (HÜBAK-22247).

New Record of Predatory Thrips Aeolothrips wittmeri

The first author would like to thank Assist. Prof. Dr. Musa Büyük (Dicle University, Diyarbakır Agriculture Vocational School, Türkiye) for his help in the field sampling. We are grateful to anonymous reviewers for useful comments.

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Studies on Insect Pollinator Fauna and Behaviour of Honeybees in Indian Mustard, [*Brassica juncea* (L.) Czern. and Coss]

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ABSTRACT

Understanding honeybee foraging behaviour optimizes pollinator usage, increasing crop yield, quality, and ecosystem biodiversity, benefiting mustard and other crops. The present investigations were carried out in the experimental field of mustard crop and the apiary at RVSKVV- Krishi Vigyan Kendra, Morena, Madhya Pradesh, during 2021-22. Results revealed that a total of 36 species belonging to 8 orders viz., Hymenoptera (12), Diptera (9), Lepidoptera (6), Coleoptera (3), Hemiptera (2), Orthoptera (2), Neuroptera (1) and Odonata (1). The comparative mean abundance among the various bees appearing on mustard flowers revealed that *Apis mellifera* (15.29 bees/m²/5 min) was the predominant visitor followed by *A. dorsata* (11.57 bees/m²/5 min) and *A. cerana indica* (6.81 bees/m²/5 min). It was found that *A. mellifera*, *A. dorsata* and *A. indica* constituted 45.40, 34.37 and 20.23 per cent, respectively. The maximum mean foraging speed during different day hours varied from 3.33 to 8.66 seconds for *A. cerana indica* 1200 hrs to 1600 hrs, respectively and for *A. mellifera* it varied from 1.46 to 3.66 seconds at 1800 hrs to 1400 hrs, respectively. Additionally, the highest mean foraging rate during different day hours varied from 10.71 to 21.43 flowers visited/min for *A. mellifera* during 1800 to 1200 hrs, respectively and the mean foraging rate was lowest for *A. cerana indica* varied from 5.14 to 12.29 flowers visited/min during 1800 to 1200 hrs, respectively.

Keywords: *Apis mellifera*, *Apis dorsata*, *Apis cerana indica*, pollinators diversity, foraging speed, foraging rate, bee abundance, bee activity, Hymenoptera

Bijarniya, M., Yadav, A.S., & Naveen (2024). Studies on insect pollinator fauna and behaviour of honeybees in Indian mustard, [*Brassica juncea* (L.) Czern. and Coss] . *Journal of the Entomological Research Society*, 26(1), 39-52.

Received: June 13, 2023

Accepted: March 12, 2024

INTRODUCTION

Indian mustard, *Brassica juncea* (L.) Czern & Coss., commonly referred to as *sarson* or *rai* (Hindi) is the oldest cultivated dicotyledonous angiosperm plant belonging to Brassicaceae or Cruciferae family which is generally pollinated by insects (Bhowmik, Mitra, & Bhadra, 2014). It is the world's second-largest oilseed crop after groundnut with its distribution in China, Canada, India, Australia, France, Germany, United Kingdom, etc. In India, mustard is grown at an area of 66.99 Mha, with an output of 102.10 million tonnes and productivity of 1524 kg/ha with its distribution in Rajasthan, Haryana, Madhya Pradesh, Uttar Pradesh and West Bengal (Anonymous, 2021). In several cross-pollinated crops like mustard, external bioagents are required to accomplish this process and pollination in these crops so as to increase the seed production significantly. Honeybees is considered one of the effective, cheapest and eco-friendly input methods for triggering the crop yield both qualitatively and quantitatively. Pollination through honeybees ensures uniform maturity and early harvest of the crop (Anil, 2015). The mustard bloom was visited by 88 insects from 63 species belonging to 31 families and 9 orders. Order Hymenoptera formed a higher percentage of the insect visitors in scan sampling (Devi et al, 2017 and Kunjwal, Kumar, & Khan, 2014).

Further, foraging behaviour of different species of honeybee shows the efficiency of their activity in the field. Rathi & Sihag (1993) revealed that the honey bee species were more active from 11.00 h to 14.00 h, whereas, the solitary bees were more active later (12.00 to 15.00 h). Painkra & Shaw (2016) observed maximum visitation of *Apis cerana* at 1100 hrs (66.06 bees/5 min/m²). The maximum foraging activity of *Apis dorsata* was observed at 1100 hrs. (11.75 bees/5 min/m²) whereas, the lowest was observed at 1700 hrs. (0.50 bee/5 min/m²). The higher foraging activity of *Apis florea* was noticed at 1300 hrs. (4.00 bees/5 min/m²) and was found least at 0900 hrs (0.56 bee/5 min/m²). Nagpal, Yadav, & Singh, (2020) revealed that *Apis florea* had the longest average time spent per flower (6.08 sec), followed by *Apis dorsata* (3.41 sec), *Apis mellifera* (2.60 sec), and *Apis cerana indica* (2.33 sec). This indicates that *A. cerana indica* is the fastest of all the *Apis* species, whereas *A. florea* is comparatively slower, maybe as a result of the length of its tongue. Further, the average time spent per flower of various *Apis* spp. over day periods varied from 3.25 to 4.20 seconds. *Apis mellifera*, *Apis dorsata*, and *Apis cerana indica* spent the least amount of time per flower (2.02 seconds, 2.38 seconds and 2.97 seconds, respectively) at 1200-1400 h, but *Apis florea* lowest amount of time spent per flower was (4.23 sec) at 0800-1000 h on *Brassica juncea*. The timing of honeybee foraging is crucial for efficient pollination. Different species of honeybees are most active during their specific times of the day when flowers are open, ensuring optimal flower visitation. It is therefore possible to employ the most appropriate species of honeybees by understanding the visitation timing of honeybees. Therefore, the present research in Indian mustard ecosystem will be of significance to understand the importance of pollinators fauna, foraging behaviour of different honeybee species in the study area with the aim to increase the biodiversity, crop yield and quality which ultimately contributes to the

conservation of major pollinators fauna. So, in light of the facts and information stated above, the purpose of this study was to develop an understanding of the diversity of insects pollinating Indian Mustard and the knowledge of foraging behaviour of different species of honeybees to develop better understanding of the most efficient pollinator.

MATERIAL AND METHODS

The present investigations were carried out in the assigned experimental field of mustard crop and the apiary at RVSKVV- Krishi Vigyan Kendra, Morena, Madhya Pradesh, during 2021-22. The healthy seeds of mustard variety RVM- I were sown on 24-10-2021 at 5 cm depth at a row distance of 45 cm and plant to plant of 15 cm apart with the plot size of 6.0 m x 3.0 m by following recommended package of practices.

For the observations of the insects that visited and occurred on plants were surveyed using the direct count observation approach. During the peak flowering season observations were made for distinct groups of pollinators visiting the mustard crop field in each square meter area from three sites. To estimate the pollinator fauna as well as the predominance (in percent value) of a given group, the observed data were aggregated group-wise. The dominance per cent was computed by multiplying the total number of genera seen by the number of species observed in each insect order.

For the observations on the foraging behaviour of honey bees, three species were taken into consideration viz., *Apis mellifera*, *Apis dorsata*, *Apis cerana indica*. The observations began at 10% flowering and continued until the flowering phase was completed. The plots were replicated thrice for recording the observations. The data were recorded at weekly intervals on the number of honeybees visiting in each square meter area from randomly marked five spots in each of the replicated plots for five minutes at two hourly intervals from 0800 to 1800 hours. The amount of time that different honey bee species spent on live flowers and the number of flowers visited per minute was also recorded from randomly marked five spots in each of the replicated plots for five minutes at two hourly intervals from 0800 to 1800 hours. The data was averaged over time, weekly and even across species to determine which group and when it dominated over the other species. Furthermore, a two-way analysis of variance was performed on different species of honeybees to see how weeks and time hours of the day influenced their foraging behavior, as well as whether these two factors interact.

RESULTS

Diversity of insect pollinators of Indian mustard

The experimental field was observed carefully during the study period and the insect pollinators visiting the mustard crop (*Brassica juncea* L.) were collected. Unknown insects were captured using appropriate collection protocols, then identified with the help of insect collections and literature available in the department of Entomology, Central Library and with the help of members of the advisory committee. All the

identified insect visitors along with their scientific names and systemic positions are listed in Table 1.

Table 1. List of insect pollinators' fauna of mustard crop observed and collected during Rabi 2021-22 flowering season.

S. No.	Common Name	Scientific Name	Family	Order
1	European honey bee	<i>Apis mellifera</i> Linnaeus	Apidae	Hymenoptera
2	Rock bee	<i>Apis dorsata</i> Fabricius		
3	Indian honeybee	<i>Apis cerana indica</i> Fabricius		
4	Little honeybee	<i>Apis florea</i> Fabricius		
5	Stingless bee	<i>Tetragonula laeviceps</i> Smith		
6	Small carpenter bee	<i>Ceratina sexmaculata</i> Smith		
7	Leaf cutter bee	<i>Megachile disjuncta</i> Fabricius	Megachilidae	
8	Carpenter bee	<i>Xylocopa iridipennis</i> Lepeletier	Xylocopidae	
9	Digger bee	<i>Anthophora</i> sp.	Anthophoridae	
10	Scolid wasp	<i>Scolia (Discolia) binolata</i> Fabricius	Scolidae	
11		<i>Campsomeriella</i> sp.		
12	Digger wasps	<i>Sphex</i> sp.	Sphecidae	
13	Syrphid fly	<i>Syrphus corollae</i> Fabricius	Syrphidae	Diptera
14		<i>Episyrphus veltatus</i> De Geer		
15	Hover fly	<i>Episyrphus balteatus</i> De Geer		
16		<i>Metasyrphus confrater</i> Wiedemann		
17	Drone fly	<i>Eristalis</i> sp.		
18	House fly	<i>Musca domestica</i> Linnaeus		
19	Orientalis house fly	<i>Atherigona orientalis</i> (Schiner)		
20	Tephritid fruit fly	<i>Bactrocera</i> sp.	Tephritidae	
21	Blow flies	<i>Calliphora vicina</i> Robineau-Desvoidy	Calliphoridae	
22	Green stink bug	<i>Nezara viridula</i> Linnaeus	Pentatomidae	Hemiptera
23	Shield bug	<i>Bagrada hilaris</i> Burmeister		
24	Green lacewing	<i>Chrysoperla carnea</i> (Steph.)	Chrysopidae	Neuroptera
25	Lady bird beetle	<i>Coccinella septempunctata</i> Linnaeus	Coccinellidae	Coleoptera
26		<i>Cheilomenes sexmaculata</i> Fabricius		
27	Egyptian alfalfa weevil	<i>Hypera brunneipennis</i> Boheman	Curculionidae	
28	Dragon fly	<i>Hemianax ephippiger</i> Burmister	Aeshnidae	Odonata
29	Pea blue butterfly	<i>Lampides boeticus</i> Linnaeus	Lycaenidae	Lepidoptera
30	Beet armyworm	<i>Spodoptera exigua</i> Hubner		
31	Cabbage butterfly	<i>Pieris brassicae</i> Linnaeus	Pieridae	
32	Oriental hornet	<i>Vespa orientalis</i> Linnaeus		
33	Paper wasp	<i>Polistes gallicus</i> Linnaeus	Vespidae	
34	Yellow jacket	<i>Vespula squamosa</i> Drury		
35	American bird grasshopper	<i>Schistocerca americana</i> Drury	Acrididae	Orthoptera
36	Broad-tipped katydid	<i>Neoconocephalus</i> sp.	Tettigoniidae	

A total of 36 species belonging to 8 orders viz., Hymenoptera (12), Diptera (9), Lepidoptera (6), Coleoptera (3), Hemiptera (2), Orthoptera (2), Neuroptera (1) and Odonata (1) visited mustard flowers. The Hymenopteran visitors comprising of 12 species belonging to 6 families. These species of insect pollinators were followed in order of their diversity by Dipterans comprising 9 species belonging to 4 families. The third most diverse insect order was Lepidoptera comprising 6 species belonging to 3 families. The next diverse insect order was Coleoptera comprising 3 species belonging to 2 families. The least diversified orders were Hemiptera (comprising 2 species) and

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Orthoptera (comprising 2 species from 2 families) and Neuroptera and Odonata each comprising single species. The results also revealed that Hymenopterans were the most abundant pollinators (33.33 per cent), followed by the orders Diptera (25.00 per cent), Lepidoptera (16.67 per cent), and Coleoptera (8.33 per cent) throughout the flowering season of the mustard crop, as shown in Fig. 1. The abundance of Orthoptera and Hemiptera was found to be the same, at 5.56 per cent. The orders Neuroptera and Odonata have the least pollinators, accounting for 2.78 per cent of the total.

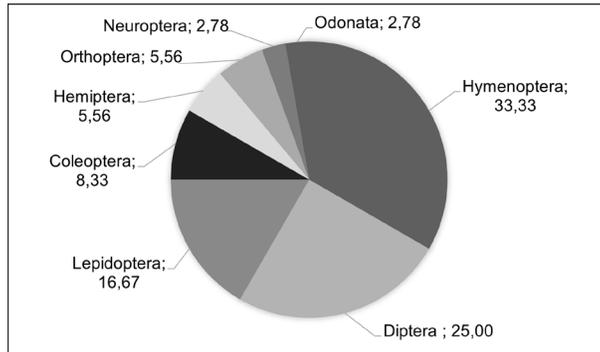


Figure 1. Per cent dominance of insect pollinators' fauna of mustard crop observed during Rabi 2021-22 flowering season.

Foraging Behaviour of Honey Bees in Mustard Crop

Bee abundance of different bee species

Observations on the abundance of different species of honey bee viz., *Apis mellifera*, *Apis dorsata*, *Apis cerana indica* on the flowers of the mustard crop are presented in Table 2. The abundance was recorded in the number of honeybees of a particular species visiting a 1 m² area of the field in 5 minutes. In general, due to very low temperatures in the morning hours in the weeks of December 2021 and January 2022, there was no honey bee activity at 0800 hrs all through the study period. However, the very negligible activity of different honey bee species was observed at 1800 hrs in the evening also.

Observations on the *Apis mellifera* abundance at weekly basis indicated that minimum abundance (10.67 bees/m²/5 min) was recorded during the first (24th December) flowering week and increased gradually with consecutive weeks up to the peak period of flowering, i.e., 4th week (15th January) with 20.50 bees/m²/5 min and later started decreasing with the cessation of flowering in the mustard crop, i.e., 7th week (5th February) with 10.83 bees/m²/5 min. The maximum abundance of *A. mellifera* bees during different day hours was reported around 1200 hrs (25.14 bees/m²/5 min), which differs considerably from typical visits at other times of the day and at 1800 hours (7.00 bees/m²/5 min), *A. mellifera* visits were at their lowest. Observations on the *Apis dorsata* abundance at weekly basis indicated that minimum abundance (5.83 bees/m²/5 min) was recorded during the first (24th December) flowering week and increased

gradually with consecutive weeks up to the peak period of flowering, i.e., 4th week (15th January) with 17.67 bees/m²/5 min and later started decreasing with the cessation of flowering in the mustard crop, i.e., 7th week (5th February) with 8.17 bees/m²/5 min. The maximum abundance of *A. dorsata* bees during different day hours was reported around 1400 hrs (19.71 bees/m²/5 min), which differs considerably from typical visits at other times of the day and at 1800 hours (3.86 bees/m²/5 min), *A. dorsata* visits were at their lowest. Observations on the *Apis cerana indica* abundance at weekly basis indicated that minimum abundance (3.50 bees/m²/5 min) was recorded during the first (24th December) flowering week and increased gradually with consecutive weeks up to the peak period of flowering, i.e., 4th week (15th January) with 9.50 bees/m²/5 min and later started decreasing with the cessation of flowering in the mustard crop, i.e., 7th week (5th February) with 4.83 bees/m²/5 min. The maximum abundance of *A. cerana indica* bees during different day hours was reported around 1000 hrs (13.14 bees/m²/5 min), which differs considerably from typical visits at other times of the day and at 1600 hrs (7.57 bees/m²/5 min), *A. cerana indica* visits were at their lowest. The comparative abundance of different bee species on the mustard crop at varied time intervals are shown in Table 3. The data revealed a significant difference and found that *A. mellifera*, *A. dorsata* and *A. indica* constituted 45.40, 34.37 and 20.23 per cent, respectively. Bees were most abundant during 1400 hrs (17.86 bees/m²/5 min) followed by the number of bees during 1200 hrs, 1000 hrs, 1600 hrs and 1800 hrs (17.14, 16.29, 12.43 and 3.62 bees/m²/5 min). As far as the abundance of different bee species at six-hour slots is concerned, it can be seen from Table 3 that the visits of different bee species varied greatly. The population density of various bees declined sharply by 1800 hrs out of three different bee species recorded during the period of investigation. The three *Apis* species were found to be fairly abundant.

Foraging speed of different bee species

Observations on the foraging speed of different honey bee species viz., *Apis mellifera*, *Apis dorsata*, *Apis cerana indica* on the flowers of the mustard crop are presented in Table 4. The foraging speed was recorded in terms of time spent (seconds) per flower. In general, due to very low temperatures in the morning hours in the weeks of December 2021 and January 2022, there was no honey bee foraging at 0800 hrs all through the study period. However, the very negligible activity of different honey bee species was observed at 1800 hrs in the evening also.

Data on the foraging speed of *A. mellifera* recorded during different weeks revealed that the maximum foraging speed of *A. mellifera* (2.95 sec) was in the fourth week and minimum foraging speed was in first week (1.49 sec) of the flowering season. As far as times of the day were concerned, the mean foraging speed of *A. mellifera* bees over different day hours revealed that maximum time spent per flower (3.66 sec) was recorded at 1400 hrs and minimum time spent per flower was recorded at 1800 h (1.46 sec). Data on the foraging speed of *A. dorsata* recorded during different weeks revealed that the maximum time spent per flower (4.06 sec) was in the fourth week and minimum foraging speed was in first week (2.49 sec) of the flowering season.

As far as times of the day were concerned, the mean foraging speed of *A. dorsata* bees over different day hours revealed that the maximum time spent per flower (6.16 sec) was recorded at 1600 hrs and minimum time spent per flower was recorded at 1800 h (1.39 sec). Data on the foraging speed of *Apis cerana indica* recorded during different weeks revealed that maximum time spent per flower (4.49 sec) was in the fourth week and minimum time spent per flower was recorded in first week (3.49 sec) of the flowering season. As far as times of the day were concerned, the mean foraging speed of *A. cerana indica* bees over different day hours revealed that the maximum time spent per flower (8.66 sec) was recorded at 1600 hrs and minimum time spent per flower was recorded at 1200 hrs (3.33 sec). The foraging speed of all three bee species on the mustard crop at varied time intervals revealed a significant difference as shown in Table 5. It was found that *A. mellifera*, *A. dorsata* and *A. indica* constituted 2.14, 3.14 and 3.91 seconds per flower. Bees spent maximum time during 1600 hrs (5.90 seconds/ flower) followed by 5.14, 3.54, 2.83 and 0.95 seconds per flower at 1400 hrs, 1000 hrs, 1200 hrs and 1800 hrs, respectively. Hence, it was found to be the least effective in pollination as it spent the highest time per flower.

Foraging rate of different bee species

Observations on the foraging rate of different species of honey bee on the flowers of the mustard crop are presented in Table 6. The foraging speed was recorded in terms of the number of flowers visited per minute. In general, due to very low temperatures in the morning hours in the weeks of December 2021 and January 2022, there was no honey bee activity at 0800 hrs all through the study period. However, the very negligible activity of different honey bee species was observed at 1800 hrs in the evening also.

Observations on the foraging rate of *A. mellifera* recorded during different weeks over the times revealed that the mean weekly foraging rate ranged between 6.67 to 20.33 flowers visited/min corresponding to the first week of the blooming period and fourth week of the blooming period which was also its peak and it gradually decreased to 8.33 flowers visited /min as flowering came to an end (seventh week). At 1200 hours, *A. mellifera* had the highest foraging rate (21.43 flowers visited/min) throughout different day hours and lowest foraging rate was recorded at 1800 hours (10.71 flowers visited/min). Observations on the foraging rate of *A. dorsata* recorded during different weeks over the times revealed that the mean weekly foraging rate ranged between 3.33 to 16.17 flowers visited/min corresponding to the first week of the blooming period and fourth week of the blooming period which was also its peak and it gradually decreased to 5.00 flowers/min as flowering came to an end (seventh week). At 1200 hours, *A. dorsata* had the highest foraging rate (16.43 flowers visited/min) throughout different day hours and lowest foraging rate was recorded at 1800 hours (6.57 flowers visited/min). Observations on the foraging rate of *A. cerana indica* recorded during different weeks over the times revealed that the mean weekly foraging rate ranged between 1.17 to 10.67 flowers visited/min corresponding to the first week of the blooming period and fourth week of the blooming period which was also its peak and it gradually decreased to 2.50 flowers/min as flowering came to an end (seventh

week). At 1200 hours, *A. cerana indica* had the highest foraging rate (12.29 flowers visited/min) throughout different day hours and lowest foraging rate was recorded at 1600 hours (5.14 flowers visited/min). The foraging rate of all three bee species on the mustard crop at varied time intervals revealed a significant difference as shown in Table 7. It was found that *A. mellifera*, *A. dorsata* and *A. indica* constituted 13.02, 9.00 and 5.71 flowers visited per minute. Bees visited maximum flowers during 1200 hrs (16.71 flowers visited/min) followed by 12.52, 10.57, 9.90 and 5.76 flowers visited/min at 1000 hrs, 1600 hrs, 1400 hrs and 1800 hrs, respectively. Hence, it was found to be the least effective in pollination as it spent the lowest number of flowers/ min.

CONCLUSIONS AND DISCUSSION

Indian mustard flowers receive more visits from different pollinators since they exhibit different behaviours and have different tastes in floral characteristics. This increased pollination activity aids in more effective pollen movement between flowers, improving fertilisation and increasing seed set. From the above results, we can conclude that a total of 36 species belonging to 8 orders. Hymenopterans were the most abundant pollinators (33.33 per cent and 12 species), followed by the orders Diptera (25.00 per cent and 9 species), Lepidoptera (16.67 per cent and 6 species), and Coleoptera (8.33 per cent and 3 species) throughout the flowering season of the mustard crop. The abundance of Orthoptera and Hemiptera was found to be the same, at 5.56 per cent and each with 2 species. The orders Neuroptera and Odonata have the least pollinators, accounting for 2.78 per cent of the total and each with single species. The current findings are in line to Kunjwal et al, (2014) who discovered 30 species from the Hymenoptera, Diptera, Lepidoptera, and Coleoptera groups visiting mustard, *B. juncea* flowers. Hymenoptera were the most important insect pollinators among them. In comparison to other bees, *A. mellifera* was shown to be the most numerous species in all kinds of *B. juncea*. In addition, Kamel et al, (2015) discovered 21 insect pollinators from 14 families and four orders visiting canola, *B. napus* flowers. Similar results were also reported by Ahmad (2005) and Abrol (1989).

The genetic diversity of populations of Indian mustard can be increased by a variety of pollinator fauna. Indian mustard's productivity is positively impacted by a diverse pollinator fauna, resulting in encouraged and efficient cross pollination and increased agricultural production. Honeybees are extremely effective pollinators and can visit a lot of flowers in one foraging trip. Since they are plentiful, more bees will visit mustard flowers, which promotes efficient and effective pollen transfer between blooms. This greater fertilisation and higher seed set as a result of the increased pollination efficiency contribute to quantifiable gains in mustard production. As in the present study, the comparative mean abundance among the various bees appearing on mustard flowers revealed that *A. mellifera* (15.29 bees/m²/5 min) was the predominant visitor followed by *A. dorsata* (11.57 bees/m²/5 min). *A. cerana indica* (6.81 bees/m²/5 min) was the least abundant visitor of mustard flowers. It was found that *A. mellifera*, *A. dorsata* and *A. indica* constituted 45.40, 34.37 and 20.23 per cent,

respectively. However, bees were most abundant during 1400 hrs (17.86 bees/m²/5 min) and least abundant during 1800 hrs (3.62 bees/m²/5 min). The current results correspond the previous findings by Singh, Dubey, Rana, & Chandrakar (2018), who found that the order Hymenoptera contributed the most percent relative abundance and average insect population, and honeybee activity was maximum at 1200 h and 1500 h, respectively. The abundance and diversity of various insect pollinators on mustard (*Brassica juncea*) were researched by Goswami & Khan (2014) and reported that the mustard bloom was visited by 19 insect pollinators from the two orders viz., Hymenoptera and Diptera, with Hymenopterans having the maximum abundance (percent of insect/ m²/2 min), followed by Dipterans and others. The most frequent Hymenopterans were honeybees (*Apis* bees), followed by non-*Apis* bees and scolid wasps. In contrast to the current findings, Roy, Gayen, Mitra, & Duttagupta (2014) indicated that insect visits were most active during the middle of the day, from 12 p.m. to 2 p.m. The abundance of *A. dorsata* was the highest (18%) among the three honey bee species, followed by *A. cerana* (15%) and *A. florea* (12%). Similar results were also reported by Khatkar (1996) and Priti (1996).

Faster foraging honeybees promote both qualitative and quantitative growth in mustard. As in the present investigation, the mean foraging speed during different hours of the day varied from 1.46 to 3.66 seconds for *A. mellifera* at 1800 hrs to 1400 hrs, respectively which was the lowest among the three species. In the end, the mean foraging speed during different hours of the day varied from 3.33 to 8.66 seconds for *A. cerana indica* 1200 hrs to 1600 hrs, respectively which was the maximum time spent per flower. The current findings are in support with the findings of Nagpal et al, (2020) who found that the average duration spent per flower by different *Apis* spp. throughout different day periods ranged from 3.25 to 4.20 seconds. Over various day periods, the average duration spent per flower by several *Apis* spp. ranged from 3.25 to 4.20 seconds. At 1200-1400 h on *Brassica juncea*, *Apis mellifera*, *Apis dorsata*, and *Apis cerana indica* spent the least amount of time per flower (2.02 seconds, 2.38 seconds, and 2.97 seconds, respectively). The present results are also supported by the previous studies conducted by Painkra & Shaw (2016) and Manhare, Painkra, Painkra, & Bhagat (2017).

Honeybees that forage more frequently can visit more flowers in a given amount of time. The probability of efficient pollen transfer between flowers rises due to this floral visitation efficiency, which boosts pollination rates. Higher foraging rates enable honeybees to travel farther and discover more flowering plants that ultimately aids in synchronising the flowering of mustard plants and increases flower density. As in the present investigation, the mean foraging rate during different hours of the day varied from 10.71 to 21.43 flowers visited/min for *A. mellifera* during 1800 to 1200 hrs, respectively which was the highest among the three species. Hence, it was found to be most effective in pollination as it spent the highest number of flowers/min. In the end, the mean foraging rate during different hours of the day varied from 5.14 to 12.29 flowers visited/min for *A. mellifera* during 1800 to 1200 hrs, respectively being the lowest. The current findings are in agreement with those of the other researchers.

According to Choudhary & Singh (2007) the mean foraging rate of *A. mellifera* (5.0) was greater than that of *A. dorsata* (3.92) and *A. florea* (2.91). Because of the low temperature and fog water deposited on the floral parts, *A. mellifera* was not seen on coriander flowers until 07:00 h, but its population grew afterwards. At 14:00 h, the greatest foraging rate of *A. mellifera* was reported (13.6 umbels visited min⁻¹). This species visited an average of 1 plant and a maximum of 3.10 plants per five minutes. Also, Kumar, Singh, & Chand (2003), found that the foraging rate (number of capitulum visited by bees/minutes) indicated that *Apis mellifera* visited a larger number of capitulum (12.85) as compared to *Apis cerana indica* (10.98). For both species, the foraging rate was highest at 1:00 p.m. and lowest at 10:00 a.m.

ACKNOWLEDGEMENT

The authors greatly acknowledge the Head, Department of Entomology, College of Agriculture, Gwalior, Madhya Pradesh; Associate Director Research, Zonal Agricultural Research Station, Morena and Senior Scientist and Head, Krishi Vigyan Kendra, Morena, Madhya Pradesh for providing necessary facilities and their constant support throughout the study period.

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Table 2. Abundance of different honeybee species on the flowers of mustard under open pollination during Rabi 2021-22.

SMW	Bee Abundance of <i>Apis mellifera</i> (No. of bees/ m ² / 5min) Hours of the day							Bee Abundance of <i>Apis dorsata</i> (No. of bees/ m ² / 5min) Hours of the day							Bee Abundance of <i>Apis cerana indica</i> (No. of bees/ m ² / 5min) Hours of the day						
	800	1000	1200	1400	1600	1800	Mean	800	1000	1200	1400	1600	1800	Mean	800	1000	1200	1400	1600	1800	Mean
52	0.00 (1.0)	13.00 (3.74)	20.00 (4.58)	17.00 (4.24)	14.00 (3.87)	10.00 (3.07)	10.67 (3.07)	0.00 (1.0)	9.00 (3.16)	8.00 (3.0)	11.00 (3.46)	7.00 (2.83)	0.00 (1.0)	5.83 (2.41)	0.00 (1.0)	8.00 (3.0)	7.00 (2.83)	6.00 (2.65)	0.00 (1.0)	3.50 (1.91)	
1	0.00 (1.0)	19.00 (4.47)	25.00 (5.1)	23.00 (4.9)	17.00 (4.24)	10.00 (3.45)	14.00 (4.02)	0.00 (1.0)	18.00 (4.36)	15.00 (4.0)	20.00 (4.58)	10.00 (3.32)	0.00 (1.0)	10.50 (3.04)	0.00 (1.0)	13.00 (3.74)	11.00 (3.46)	9.00 (3.16)	0.00 (1.0)	7.00 (2.59)	
2	0.00 (1.0)	21.00 (4.69)	26.00 (5.2)	30.00 (5.57)	18.00 (4.36)	10.00 (3.32)	17.50 (4.02)	0.00 (1.0)	22.00 (4.8)	17.00 (4.24)	24.00 (5.0)	14.00 (3.87)	8.00 (3.0)	14.17 (3.65)	0.00 (1.0)	16.00 (4.12)	13.00 (3.74)	11.00 (3.46)	10.00 (3.32)	8.33 (2.77)	
3	0.00 (1.0)	23.00 (4.9)	33.00 (5.83)	20.00 (5.74)	20.00 (4.58)	15.00 (4.0)	20.50 (4.34)	0.00 (1.0)	26.00 (5.2)	23.00 (4.9)	27.00 (5.29)	20.00 (4.58)	10.00 (3.32)	17.67 (4.05)	0.00 (1.0)	18.00 (4.36)	15.00 (4.0)	13.00 (3.74)	11.00 (3.46)	9.50 (2.93)	
4	0.00 (1.0)	19.00 (4.47)	31.00 (5.66)	30.00 (5.57)	19.00 (4.47)	13.00 (3.74)	18.67 (4.15)	0.00 (1.0)	21.00 (4.69)	18.00 (4.36)	22.00 (4.8)	16.00 (4.12)	9.00 (3.16)	14.33 (3.69)	0.00 (1.0)	17.00 (4.24)	14.00 (3.87)	12.00 (3.61)	10.00 (3.32)	8.83 (2.84)	
5	0.00 (1.0)	15.00 (4.0)	24.00 (5.0)	22.00 (4.8)	17.00 (4.24)	11.00 (3.75)	14.83 (3.75)	0.00 (1.0)	17.00 (4.24)	14.00 (3.87)	19.00 (4.47)	12.00 (3.61)	0.00 (1.0)	10.33 (3.03)	0.00 (1.0)	11.00 (3.46)	9.00 (2.83)	7.00 (2.83)	0.00 (1.0)	5.87 (2.38)	
6	0.00 (1.0)	13.00 (3.74)	17.00 (4.24)	19.00 (4.47)	16.00 (4.12)	0.00 (3.1)	10.83 (3.1)	0.00 (1.0)	14.00 (3.87)	12.00 (3.61)	15.00 (4.0)	8.00 (3.0)	0.00 (1.0)	8.17 (2.75)	0.00 (1.0)	9.00 (3.16)	8.00 (2.65)	6.00 (2.65)	0.00 (1.0)	4.83 (2.24)	
Mean	0.00 (1.0)	17.57 (4.29)	25.14 (5.09)	24.71 (5.04)	17.29 (4.27)	7.00 (2.5)	-	0.00 (1.0)	18.14 (4.33)	15.29 (4.0)	19.71 (4.52)	12.43 (3.62)	3.86 (1.93)	-	0.00 (1.0)	13.14 (3.73)	11.00 (3.44)	9.14 (2.82)	7.57 (2.82)	0.00 (1.0)	

*figures in parentheses are square root transformed values.

Table 3. Comparative abundance of different honey bee species on the flowers of mustard crop under open pollination during Rabi 2021-22.

Foraging time (Hours of the day)	Bee Abundance (No. of bees/ m ² / 5min)					
	<i>Apis mellifera</i>		<i>Apis dorsata</i>		<i>Apis cerana indica</i>	
	SE(m)	C.D. (p= 0.05)	SE(m)	C.D. (p= 0.05)	SE(m)	C.D. (p= 0.05)
800	0.012	0.034	0.014	0.040	0.018	0.050
1000	0.011	0.031	0.013	0.037	0.017	0.047
1200	0.029	0.082	0.035	0.099	0.044	0.123
Mean	0.00 ± 0.00		0.00 ± 0.00		0.00 ± 0.00	
800	17.57 ± 1.49		18.14 ± 2.11		13.14 ± 1.50	
1000	25.14 ± 2.13		15.29 ± 1.80		11.00 ± 1.18	
1200	24.71 ± 2.24		19.71 ± 2.04		9.14 ± 1.10	
1600	17.29 ± 0.75		12.43 ± 1.74		7.57 ± 1.43	
1800	7.00 ± 2.54		3.86 ± 1.83		0.00 ± 0.00	
Mean	15.29		11.57		6.81	
% of total bees	45.40		34.37		20.23	

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Table 4: Foraging speed of different honeybee species on the flowers of mustard crop under open pollination during Rabi 2021-22.

SMW	Foraging Speed of <i>Apis mellifera</i> (sec/ flower) Hours of the day						Foraging Speed of <i>Apis dorsata</i> (sec/ flower) Hours of the day						Foraging Speed of <i>Apis cerana indica</i> Hours of the day						
	800	1000	1200	1400	1600	1800	800	1000	1200	1400	1600	1800	800	1000	1200	1400	1600	1800	
52	0.00 (1)	2.19 (1.79)	2.01 (1.73)	2.51 (1.87)	2.22 (1.79)	0.00 (1)	1.49 (1.53)	3.21 (2.05)	2.51 (1.87)	4.22 (2.28)	5.01 (2.45)	0.00 (1)	2.49 (1.78)	0.00 (1)	4.21 (2.28)	3.01 (2)	6.22 (2.69)	7.51 (2.92)	0.00 (1)
1	0.00 (1)	2.43 (1.85)	2.22 (1.79)	2.98 (1.99)	2.59 (1.89)	0.00 (1)	1.70 (1.59)	3.51 (2.12)	2.72 (1.93)	4.59 (2.36)	5.48 (2.55)	0.00 (1)	2.72 (1.83)	0.00 (1)	4.51 (2.35)	3.22 (2.05)	6.59 (2.75)	7.98 (3)	0.00 (1)
2	0.00 (1)	2.62 (1.91)	2.43 (1.85)	3.82 (2.2)	3.15 (2.04)	2.54 (1.88)	2.43 (1.81)	3.62 (2.15)	2.93 (1.98)	5.15 (2.48)	6.32 (2.71)	3.14 (2.03)	3.53 (2.06)	0.00 (1)	4.62 (2.37)	3.43 (2.1)	7.15 (2.85)	8.82 (3.13)	0.00 (1)
3	0.00 (1)	2.92 (1.98)	2.62 (1.91)	5.97 (2.64)	3.42 (2.1)	2.75 (1.94)	2.95 (1.93)	3.92 (2.22)	3.12 (2.03)	5.42 (2.53)	8.47 (3.08)	3.42 (2.1)	4.06 (2.16)	0.00 (1)	4.92 (2.43)	3.62 (2.15)	7.42 (2.9)	10.97 (3.46)	0.00 (1)
4	0.00 (1)	2.59 (1.89)	2.53 (1.88)	4.12 (2.26)	3.22 (2.05)	2.55 (1.88)	2.50 (1.83)	3.59 (2.14)	3.03 (2.01)	5.22 (2.49)	6.62 (2.76)	3.16 (2.04)	3.60 (2.07)	0.00 (1)	4.59 (2.36)	3.53 (2.13)	7.22 (2.87)	9.12 (3.18)	0.00 (1)
5	0.00 (1)	2.42 (1.85)	2.39 (1.84)	3.51 (2.12)	2.99 (2)	2.99 (1.84)	2.28 (1.78)	3.71 (2.17)	2.89 (1.97)	4.99 (2.45)	6.01 (2.65)	0.00 (1)	2.93 (1.87)	0.00 (1)	4.71 (2.39)	3.39 (2.1)	6.99 (2.83)	8.51 (3.08)	0.00 (1)
6	0.00 (1)	2.25 (1.8)	2.14 (1.77)	2.74 (1.93)	2.57 (1.89)	0.00 (1)	1.62 (1.57)	3.41 (2.1)	2.64 (1.91)	4.57 (2.36)	5.24 (2.5)	0.00 (1)	2.64 (1.81)	0.00 (1)	4.41 (2.33)	3.14 (2.03)	6.57 (2.75)	7.74 (2.96)	0.00 (1)
Mean	0.00 (1)	17.42 (1.87)	16.34 (1.83)	25.65 (2.15)	20.16 (1.97)	10.23 (1.51)	-	24.96 (2.14)	19.84 (1.96)	34.16 (2.42)	43.15 (2.67)	9.71 (1.45)	-	0.00 (1)	31.96 (2.36)	23.34 (2.08)	48.16 (2.81)	60.65 (3.1)	0.00 (1)

*figures in parentheses are square root transformed values.

Factors	<i>Apis mellifera</i>		<i>Apis dorsata</i>		<i>Apis cerana indica</i>	
	SE(m)	C.D. (p= 0.05)	SE(m)	C.D. (p= 0.05)	SE(m)	C.D. (p= 0.05)
SMW	0.027	0.076	0.024	0.068	0.023	0.065
Time	0.025	0.070	0.022	0.063	0.021	0.061
SMW x Time	0.066	0.186	0.059	0.167	0.057	N/A

Table 5. Comparative foraging speed of different honey bee species on the flowers of mustard crop under open pollination during Rabi 2021-22.

Foraging time (Hours of the day)	Foraging Speed (sec/ flower)		
	<i>Apis mellifera</i>	<i>Apis dorsata</i>	<i>Apis cerana indica</i>
800	0.00 ± 0.00	0.00 ± 0.00	0.00
1000	2.49 ± 0.09	3.57 ± 0.08	3.54
1200	2.33 ± 0.08	2.83 ± 0.08	2.83
1400	3.66 ± 0.44	4.88 ± 0.16	5.14
1600	2.88 ± 0.16	6.16 ± 0.44	5.90
1800	1.46 ± 0.52	1.39 ± 0.65	0.95
Mean	2.14	3.14	3.91

Table 6. Foraging rate of different honeybee species on the flowers of mustard crop under open pollination during Rabi 2021-22.

SMW	Foraging Rate of <i>Apis mellifera</i> (No. of flower/ min) Hours of the day						Foraging Rate of <i>Apis dorsata</i> (No. of flower/ min) Hours of the day						Foraging Rate of <i>Apis cerana indica</i> (No. of flower/ min) Hours of the day								
	800	1000	1200	1400	1600	1800	Mean	800	1000	1200	1400	1600	1800	Mean	800	1000	1200	1400	1600	1800	Mean
52	0.00 (1)	9.00 (3.16)	13.00 (3.74)	7.00 (2.83)	11.00 (3.46)	0.00 (1)	6.67 (2.53)	0.00 (1)	6.00 (2.65)	8.00 (3)	4.00 (2.24)	2.00 (1.73)	0.00 (1)	3.33 (2.08)	0.00 (1)	2.00 (1.73)	4.00 (2.24)	1.00 (1.41)	1.00 (1)	0.00 (1)	1.17 (1.4)
1	0.00 (1)	14.00 (3.87)	21.00 (4.69)	13.00 (3.74)	17.00 (4.24)	0.00 (1)	10.83 (3.09)	0.00 (1)	11.00 (3.46)	16.00 (4.12)	9.00 (3.16)	8.00 (3)	0.00 (1)	7.33 (2.89)	0.00 (1)	8.00 (3)	12.00 (3.61)	6.00 (2.65)	5.00 (2.45)	0.00 (1)	5.17 (2.28)
2	0.00 (1)	17.00 (4.24)	26.00 (5.2)	16.00 (4.12)	22.00 (4.8)	18.00 (4.36)	16.50 (3.95)	0.00 (1)	17.00 (4.24)	21.00 (4.69)	12.00 (3.61)	11.00 (3.46)	0.00 (1)	14.00 (3.87)	0.00 (1)	13.00 (3.74)	17.00 (4.24)	10.00 (3.32)	8.00 (3)	0.00 (1)	8.00 (2.72)
3	0.00 (1)	22.00 (4.8)	30.00 (5.57)	19.00 (4.47)	27.00 (5.29)	24.00 (5)	20.33 (4.35)	0.00 (1)	22.00 (4.8)	25.00 (5.1)	17.00 (4.24)	14.00 (3.87)	0.00 (1)	19.00 (4.47)	0.00 (1)	18.00 (4.36)	21.00 (4.69)	14.00 (3.87)	11.00 (3.46)	0.00 (1)	10.67 (3.06)
4	0.00 (1)	16.00 (4.12)	25.00 (5.1)	15.00 (4)	22.00 (4.8)	19.00 (4.47)	16.17 (3.92)	0.00 (1)	17.00 (4.24)	20.00 (4.58)	11.00 (3.32)	10.00 (3.16)	0.00 (1)	13.00 (3.58)	0.00 (1)	15.00 (4)	16.00 (4.12)	11.00 (3.46)	7.00 (2.83)	0.00 (1)	8.17 (2.74)
5	0.00 (1)	12.00 (3.61)	19.00 (4.47)	11.00 (3.46)	18.00 (4.36)	14.00 (3.87)	12.33 (3.46)	0.00 (1)	12.00 (3.61)	14.00 (3.87)	9.00 (2.65)	6.00 (2.45)	0.00 (1)	6.83 (2.8)	0.00 (1)	9.00 (3.16)	10.00 (3.32)	4.00 (2.24)	3.00 (2)	0.00 (1)	4.33 (2.12)
6	0.00 (1)	11.00 (3.46)	16.00 (4.12)	10.00 (3.32)	13.00 (3.74)	0.00 (1)	8.33 (2.77)	0.00 (1)	8.00 (3)	11.00 (3.46)	6.00 (2.45)	5.00 (2.24)	0.00 (1)	5.00 (2.45)	0.00 (1)	4.00 (2.24)	6.00 (2.65)	3.00 (2)	2.00 (1.73)	0.00 (1)	2.50 (1.77)
Mean	0.00 (1)	101.00 (3.9)	150.00 (4.7)	91.00 (3.71)	130.00 (4.36)	75.00 (2.96)	-	0.00 (1)	93.00 (3.78)	115.00 (4.17)	68.00 (3.27)	56.00 (3)	46.00 (2.75)	-	0.00 (1)	9.86 (3.18)	12.29 (3.55)	7.00 (2.71)	5.14 (2.35)	0.00 (1)	0.00 (1)

*figures in parentheses are square root transformed values.

Factors	<i>Apis mellifera</i>		<i>Apis dorsata</i>		<i>Apis cerana indica</i>	
	SE(m)	C.D. (p= 0.05)	SE(m)	C.D. (p= 0.05)	SE(m)	C.D. (p= 0.05)
SMW	0.013	0.037	0.018	0.050	0.024	0.068
Time	0.012	0.034	0.016	0.046	0.022	0.063
SMW x Time	0.032	0.090	0.043	0.123	0.059	0.168

Table 7. Comparative foraging rate of different honey bee species on the flowers of mustard crop under open pollination during Rabi 2021-22.

Foraging time (Hours of the day)	Mean number of flowers visited/ min		
	<i>Apis mellifera</i>	<i>Apis dorsata</i>	<i>Apis cerana indica</i>
800	0.00 ± 0.00	0.00 ± 0.00	0.00
1000	14.43 ± 0.09	13.29 ± 0.08	9.86 ± 0.08
1200	21.43 ± 0.08	16.43 ± 0.08	12.29 ± 0.08
1400	13.00 ± 0.44	9.71 ± 0.16	7.00 ± 0.16
1600	18.57 ± 0.16	8.00 ± 0.44	5.14 ± 0.44
1800	10.71 ± 0.52	6.57 ± 0.65	0.00 ± 0.00
Mean	13.02	9.00	5.71

A New Species of the Genus *Solva* (Diptera: Xylomyidae) and First Report of *Solva javana* (Meijere, 1907) from the Tropical Forest of Andaman Island

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ABSTRACT

A new species of wood-soldier fly under the genus *Solva* Walker, 1859 (Diptera: Xylomyidae) (*Solva andamanensis* sp. n. Pramanik, Naskar & Banerjee) is described from the tropical forest of Andaman Island, India. Males can be distinguished from other species by a combination of characters, specifically the ochre yellow antenna; snow-white cylindrical palpi; yellow fore coxa, one antero-dorsal bristle in fore femur, black scutellum and black hind coxa. Male terminalia is illustrated here. Furthermore, *Solva javana* (Meijere, 1907) (Diptera: Xylomyidae) is reported for the first time from Andaman Island as well as India. The difference in habitus characters between these two species is also illustrated in this study.

Keywords: Stratiomyomorpha, Wood Soldier fly, phytosaprophagous fly, nutrient recycling, island ecosystem.

INTRODUCTION

Xylomyidae, commonly known as the Wood-soldier flies, is one of the less-studied families within the order Diptera, with only 132 reported species belonging to 4 valid genera currently known in the world (Woodley, 2011). However, this number has notably increased to 143 species according to Fachin & de Assis-Pujol (2016). Morphologically, adult flies of this family are identified by the presence of an elongated discal cell and a closed M3 cell in the wing. The adult flies are mainly found in forested areas and immature stages are phyto-saprophagous, usually developing under the bark of fallen trees (Webb, 1984). Taxonomic studies of this family are very scarce and most of the literatures date back to the 20th century. Earlier, the wood-soldier flies were considered as a subfamily under the family Stratiomyidae. Later, these flies were recognised as a separate family of the infraorder Stratiomyomorpha, as a sister-group of Stratiomyidae (Woodley, 1989). The naming of the family was a subject of discussion for a long time as indicated in the works of Webb (1984) and Nagatomi (1993). Initially, the wood-soldier flies were referred as family 'Solvidae', however Nagatomi (1975) suggested that the name 'Xylomyidae' should be used instead of 'Solvidae' after the separation of *Xylomya* Rondani, 1861 from *Solva* Walker, 1859 as a distinct genus. Brunetti (1923) provided the taxonomic key for 18 species of the genus *Xylomya* from Oriental region of which five species were new to science that were duly described by him – *X. nigra*, *X. nigriventris*, *X. inconspicua*, *X. intermedia* and *X. similis*. However, those 18 species were later shifted under the genus *Solva*. Steyskal (1947) was probably the first to describe the two major genera - *Solva* and *Xylomya* in detail. In the Catalog of the Diptera of the Oriental region, Nagatomi (1975) stated that 46 species occur in the Oriental region, of which 13 species (12 species of *Solva* and one species of *Coenomyiodes*) are found in India. Later, Yang and Nagatomi (1993) published a detailed account about the Xylomyidae of China. They introduced 25 new species to science from China which made the total species count in China 35 belonging to 3 genera. Daniels (1976, 1989) was a stalwart in research about Xylomyidae from Oceania's biogeographic zone. He provided a detailed diagnosis and taxonomic key of six species of genus *Solva* found in Australia and Papua New Guinea (Daniels, 1976). In recent times, Krivosheina et al (2015) introduced a new species of *Solva* from Sumatra (*S. richterae*) by rearing larva found in decaying wood. Krivosheina (2016a) discussed about the larval and adult diagnostic features of four species of *Xylomya* from Russia. The larval ecology and morphological features of genus *Solva* have also been studied by Krivosheina (2016b) while studying the insect successional stages in a decomposing wood.

The research on the family Xylomyidae in the Andaman and Nicobar Islands is lacking. The overall study on Diptera is also less from these bay islands in comparison with the mainland and no separate effort has been given to the family Xylomyidae. The Andaman and Nicobar Islands are known for their luxuriant and lush green tropical rainforest with its characteristic hot and humid tropical climate. The Middle and North Andaman Islands, from where the specimens were found are mainly composed of Moist Deciduous and Wet Evergreen forest types (FSI, 2019).

MATERIALS AND METHODS

The study is based on the collection done from 7th to 26th September 2022 in Andaman islands. Sampling was primarily done by sweep netting in the forested patches where there is above-ground litter layer and decaying wood. Collected specimens were preserved in 70% ethyl alcohol for further study. The genitalia was dissected from the specimens and cleared with water-diluted potassium hydroxide (KOH) pellets before being neutralized with acetic acid. A Leica EZ4 HD stereo microscope was used for the identification of the specimens. Images were taken with a Leica M205A stereo iso-microscope coupled with a LEICA DFC 500 camera and software Leica Application Suite LAS V3.6. The images were edited with Adobe Photoshop CC version 13.0.1. All measurements and incorporated scale bars of the images are in millimetres (mm) [1 mm = 0.001 m]. The field photographs were captured using a Nikon D7200 Digital SLR camera coupled with Nikkor 18 – 140 mm f/3.5 – 5.6 G ED VR lens.

Body length is measured from the base of the antennae to the tip of the abdominal segment and wing length from the wing base to the wing apex. Taxonomic descriptions and terminologies are based on the format of McAlpine (1981). The holotype and the paratypes are deposited in the National Zoological Collection of Diptera Section, Zoological Survey of India, Kolkata.

RESULTS

This article deals with 2 species of the genus *Solva* – *Solva andamanensis* sp. n. (Fig. 1) and *Solva javana* (Meijere, 1907) (Fig. 2). The following is a detailed taxonomic account of the 2 species.



Figure 1. Field photograph of *Solva andamanensis* sp. n.



Figure 2. Field photograph of *Solva javana* (Meijere, 1907).

Order Diptera Linnaeus, 1758

Family Xylomyidae Verrall, 1901

Genus *Solva* Walker, 1859

Solva andamanensis sp. n.

Materials examined: Holotype: ♂, Adult, Sabari Forest, Bakultala, North & Middle Andaman district, 12°29'41.7" N, 92°53'54.9" E, elevation: 27 m, 21.ix.2022, coll. by D. Pramanik. Paratypes: 4 ♂, Adult, Sabari Forest, Bakultala, North & Middle Andaman district, 12°29'41.7" N, 92°53'54.9" E, elevation: 27 m, 21.ix.2022, coll. by D. Pramanik; 1 ♂, Adult, Yeratta Forest, Bakultala, North & Middle Andaman district, 12°27'43.31" N, 92°54'2.59" E, elevation: 32 m, 20.ix.2022, coll. by D. Pramanik.

Diagnosis: Male individuals have ochre yellow antenna, snow-white palpi, black scutellum with golden-yellow hairs, yellow fore coxa, black hind coxa and brownish-yellow hind femur with a dark-brown ventral streak running along the tubercles.

Description

Body length: 5.0 – 5.5 mm, Wing length: 4.6 – 4.8 mm (Fig. 3a).

Head: 2.4 – 2.6 mm in width at its maximum extent (Fig. 3b); ocellar triangle black with golden yellow hairs (Fig. 3c); eyes black; frons 0.2 mm in width, black with dense golden yellow pile in two sides leaving a small black and bare streak in the middle (Fig. 3b). Antennal scape, pedicel and flagellum all ochre yellow; scape and pedicel with minute shining silvery hairs intermixed with few black hairs (Fig. 3d); 1st flagellar segment visibly larger than the rest (almost double in length than that of 2nd or 3rd segment). Face black with very fine whitish pollinosity; palpi snow white with small shining silver hairs (Fig. 3e); labella of same colour as of antenna with shining silver hairs intermixed with very few black hairs (Fig. 3e). From lateral view, eyes covering

A New Species of the Genus Solva (Diptera: Xylomyidae)

almost entire length and width of the head leaving no such conspicuous genal and post genal region (Fig. 3f) however, yellow and silver hairs present in post genal region.



Figure 3. Morphology of *Solva andamanensis* sp. n. a) habitus, b) head (front view), c) ocelli, d) antenna, e) palpi and labia, f) head (side view).

Thorax: Entire scutum and scutellum black with golden yellow hairs (Fig. 4a). Humeral callus, notopleural stripe and base of wing pale yellow (Fig. 4b). Pleural region black with dense snow-white hairs which is very much conspicuous in sternopleuron (Fig. 4b). The snow-white pubescence also further extends to the coxae. Halteres light yellow.

Wing: Pale brownish, tip little darker, more specifically, in the region where Radial (R1, R2+3, R4 and R5) veins are meeting with the wing margin (Fig. 4c). Entire dorsal surface covered with microtrichia. The second veinlet originating from discal cell (vein M2) incomplete i.e. not reaching wing margin. Basal portion of vein A1 has short spine-like hairs. Branching point of R2+3 from R1, curved and dull coloured than rest of the vein. This makes the origin of vein R2+3 to appear somewhat broken (however, in reality, it is not broken).

Abdomen: Six tergites (T1 – T6) visible dorsally (Fig. 4d); all tergites black with light yellow pubescence throughout the length. Pubescence little denser towards the lateral sides. T1 with a membranous semi-circular spot at base, generally pale yellow to brownish. Arch of the semi-circular spot is almost touching the hind margin of T1.

Hind margins of T2 – T4 (not T1) pale yellow; T6 smaller in length (almost half) than the preceding segments. Sternites brownish yellow to deep brown with pale yellow pubescence (Fig. 4e).

Legs: Foreleg (Fig. 4f) – All segments yellow with shining snow-white pubescence from coxa to femur that is continuous with thorax pleurites; pubescence of tibia and tarsus appears light brownish when viewed at a certain angle. Femur with one antero-dorsal bristle located right at the middle of the femur. Claws dark brown. Midleg (Fig. 5a) – same as foreleg, only lacking the antero-dorsal bristle in femur. Hindleg (Fig. 5b) – Coxa black, barring a small yellowish region in posterior-distal portion just before the articulation with the trochanter. Femur brownish-yellow with pale-yellow pubescence. Presence of black small tubercles (teeth) on ventral side of the femur. A dark brown streak present on the ventral side along the tubercles covering about 2/3rd the length of the femur. Tibia brownish yellow (except the antero-ventral side) with pale yellow pubescence; antero-ventral region of tibia dark brown, more prominent in the distal half. Basitarsus yellow with shining pale-yellow pubescence; distal tarsal segments and claws dark brown.

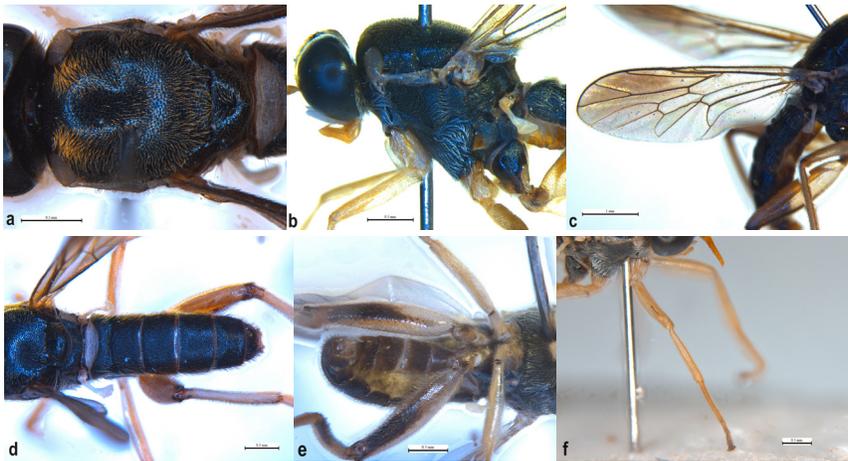


Figure 4. Morphology of *Solva andamanensis* sp. n. a) thorax (dorsal view), b) thorax (side view), c) Wing, d) abdominal tergites, e) abdominal sternites, f) fore leg.

Male terminalia: The ventral and dorsal view of the male terminalia of *Solva andamanensis* sp. n. is given (Fig. 5c and Fig. 5d respectively).

Comparative Notes: The proposed species has similarity with four species of the Oriental realm - *Solva javana* (Meijere, 1907), *Solva completa* (Meijere, 1914), *Solva nigroscutata* Meijere, 1916 and *Solva richterae* Krivosheina, 2015. These species have black streak like marking in the ventral side of hind femur like the *S. andamanensis* sp. n. But, they differ from *S. andamanensis* sp. n. in the following characteristics:- Wings of *S. andamanensis* sp. n. have brownish infuscation in the tip (Fig.4c), while *Solva javana* has comparatively clear wings (Meijere, 1907) (Fig.6c).

A New Species of the Genus Solva (Diptera: Xylomyidae)

Solva completa has distinct palpi with gradually enlarging diameter from the base onwards (Meijere, 1914) but *S. andamanensis* sp. n. has cylindrical palpi with almost the same diameter.

The first 3 antennal segments of *Solva nigroscutata* are yellow-red and the rest of the antennae are mostly black-brown (Meijere, 1916), while the antennal scape, pedicel and flagellum of *S. andamanensis* sp. n. are all ochre yellow and 1st segment of the flagellum is visibly larger than the rest.

The scutellum of the newly described *Solva richterae* is pale yellow (Krivosheina et al, 2015) but it is black in the case of *S. andamanensis* sp. n.

From the species of the Australian zoogeographical realm as described by Daniels (1976), our proposed new species has similarities with *Solva laeta* Daniels, 1977 i.e. they both have the black streak-like marking in ventral side of the hind femur. But, they differ in the following cases:

The scutellum of *Solva laeta* is yellow (black in the case of *Solva andamanensis* sp. n.).

The wings of *Solva laeta* are hyaline (dark brownish infuscation in the wing tip for *Solva andamanensis* sp. n.).

Etymology: The species' name is based on the location from where it is collected i.e. Andaman Island.



Figure 5. Morphology of *Solva andamanensis* sp. n. a) mid leg, b) hind leg, c) male genitalia (ventral view), d) male genitalia (dorsal view).

***Solva javana* (Meijere, 1907)**

Distribution: This species is reported to occur in Java Island.

Materials examined: 1 ♂, Adult, Sabari Forest, Bakultala, North & Middle Andaman district, 12°29'41.7" N, 92°53'54.9" E, elevation: 27 m, 21.09.2022, coll. by D. Pramanik.

Diagnosis: This species is described by Meijere (1907). Body length: 6.0 – 6.4 mm, Wing length: 5.0 – 5.2 mm (Fig. 6a). From our study we found it to be very similar to the previously described *Solva andamanensis* sp. n. except for 3 key differences:-

The 2nd to 8th segment of the antennal flagellum is black; the basal margin of those segments is narrowly yellowish (giving a ring-like appearance) (Fig. 6b); however, the antennal flagellum is completely ochre yellow in *Solva andamanensis* sp. n. (Fig. 3d). Fore femur in *Solva javana* lacks the antero-dorsal bristle.

Wing of *Solva javana* is comparatively less infuscated (Fig. 6c), whereas, in the case of *Solva andamanensis*, it has a distinguishing dark-brownish infuscation at the tip of the wing where the Radial veins are joining the wing margin.

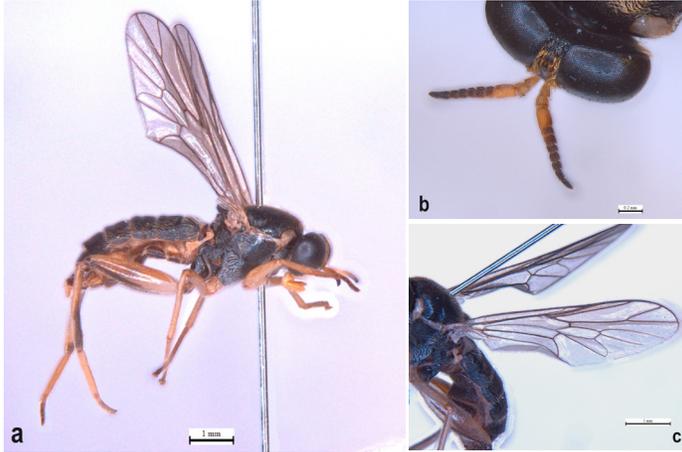


Figure 6. Morphology of *Solva javana* (Meijere, 1907). a) habitus, b) antenna, c) wing.

CONCLUSIONS

The description of a new species of *Solva* and discovery of *Solva javana* from Andaman Islands increases the species count of genus *Solva* in India from 12 to 14. The first report of *Solva javana* from Andaman Island also extends the range of this particular species which was previously reported only from Java Island. The Wood soldier flies are phyto-saprophagous in nature, in larval stages they consume dead plant materials (Webb, 1984). As the tropical forests have a carbon-rich litter layer and are a major pool of terrestrial carbon (Lalnunzira, Brearley & Tripathi, 2019), this type of habitat is apt for breeding of wood soldier flies. So, adequate survey is needed in the tropical forests of India especially in Andaman and Nicobar Islands to properly explore the Xylomyidae family.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Koustav Mukherjee, JRF, Zoological Survey of India, for assisting in the collection of specimens and taking field photographs. The authors are also thankful to Dr. C. Sivaperuman, Scientist – E & Officer-in-Charge, Andaman and Nicobar Regional Centre, ZSI for providing with essential facilities and necessary permits for conducting the survey.

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Morpho-bioecological Accounts of the Phytophagous Coccinellid Beetle, *Henosepilachna vigintioctopunctata* (Fabricius, 1775) along with Botanical Pest Management from Eastern India

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ABSTRACT

Field and laboratory investigations were carried out during 2022-23 to study the bioecology and efficacy of plant extracts for the phytophagous coccinellid, *Henosepilachna vigintioctopunctata* (F.) for understanding them properly to devise ecology based management techniques. Besides damaging the brinjal a significant population of *H. vigintioctopunctata* beetle was recorded from the solanaceous weeds like *Solanum nigrum* and *Physalis* spp. Biology of *H. vigintioctopunctata* on *S. nigrum* under laboratory conditions was conducted and the results revealed that the beetle completed its life cycle in 23.1 ± 3.5 days and the mean fecundity was 220 ± 33.0 revealing that the beetle can very successfully breed on this host plant. Morphological and taxonomical descriptions of the immature as well as adult stages of these beetles were studied and for proper identification the male genitalia was dissected. The population fluctuation of *H. vigintioctopunctata* in relation to weather parameters was also monitored under field conditions and was observed to have significant influence altogether on the pest population in brinjal but not in alternate hosts. The methanolic extracts of *Polygonum hydropiper* and tobacco decoction were found moderately effective in lowering the leaf damage by the beetle.

Keywords: *Henosepilachna vigintioctopunctata*, hosts, morphology, biology, seasonality, botanicals.

Jayashree, S. & Pal, S. (2024). Morpho-bioecological accounts of the phytophagous Coccinellid beetle, *Henosepilachna vigintioctopunctata* (Fabricius, 1775) along with botanical pest management from Eastern India. *Journal of the Entomological Research Society*, 26(1), 63-79.

Received: September 29, 2023

Accepted: March 09, 2024

INTRODUCTION

Beetles typically comes under the order coleoptera, which is the largest order in the class "Insecta". The family Coccinellidae is one of the most well-known families of Coleoptera in terms of taxonomy due to their distinctive appearance, which includes numerous species with brilliant reddish colour and black spot. It is widely known for its abundance and diversity and has over 6000 described species worldwide (Vandenberg, 2002; Akhavan, Jafari, Vafai, & Afrogheh, 2013). Coccinellid beetles, often known as ladybirds, lady bugs, or lady beetles, are frequently regarded as a representation of joy, peace, and harmony. This family comprises a number of important bio-control agents. But, members of the tribe Epilachnini under the sub family Coccinellinae are phytophagous and serious crop pests. There are over 1100 species of lady beetles in the tribe Epilachnini Mulsant, which accounts nearly 20% of the Coccinellidae (Jadwiszczak & Wgrzynowicz, 2003; Szawaryn & Tomaszewska, 2013; Tomaszewska & Szawaryn, 2016). The 27 known genera of the Epilachnini have all been redefined by Tomaszewska & Szawaryn (2016). According to the global catalogue of Epilachninae published by Jadwiszczak & Wgrzynowicz (2003) and Poorani (2004) there are 114 species of Epilachnini from the Indian Subcontinent.

According to Shirai & Katakura (1999), Islam, Islam, & Ferdous (2011), Mathur & Srivastava (1964), Dhamdhare, Koshta, & Rawat (1990), Folcia, Rodriguez, & Russo (1996), the Hadda beetle, *H. vigintioctopunctata* is reported as the key pest of many cultivated and weed plants of Solanaceae and Cucurbitaceae crops. Most of the economically significant Epilachnini species found in India, including *Henosepilachna vigintioctopunctata* (F.), *H. pusillanima* (Mulsant), *H. septima* (Dieke), and *H. vigintioctomaculata* (Motschulsky), belong to the genus *Henosepilachna* (Li & Cook, 1961). The phytophagous ladybird beetle *Epilachna vigintioctopunctata* (Fabricius) is widespread in Asia and Australia, it is a serious pest of vegetables (Khan, Islam, Rahman, & Rahman, 2000).

Both adult beetles and grubs feed on the epidermal tissue of the leaves by scrapping on the leaf surface. This results in the leaves becoming dry and eventually shed. Sometimes it is called leaf scrapping Coccinellid beetle (Imura & Ninomiya, 1978). The growth and development of plants are greatly hampered and yield is markedly reduced by attack of Epilachna beetle. Rajagopal & Trivedi (1989) have reported that Epilachna beetle may damage up to 80% of plants depending on place and season. Similarly, Rao, Chitra, & Kameswara Rao (1990) reported the damage by the beetle going beyond 30-40 percent on leaves and also nearly 50 per cent on fruits.

In vegetable production, this pest is one of the limiting factors causing both qualitative as well as quantitative losses. To compensate this pest problem, many synthetic chemical insecticides have been largely used. But, the frequent and indiscriminate use of chemical insecticides may detrimentally affect the natural enemies of Epilachna beetle. Chemical management techniques used during the crop growing season may lower the beetle population in the field, but after certain period the population eventually rebuild from the alternate hosts to the primary crop.

The interactions of *H. vigintioctopunctata* with different alternative host plants and prevailing abiotic factors should be studied and understood in detail for devising a sustainable pest management strategy with an aim to reduce the residual effect of chemicals, it can be replaced by safer and bio-degradable plant products like botanicals as pesticides. So, the present investigation was carried out to identify the life stages of the pest correctly, to determine the alternate hosts of the Hadda beetle and their seasonal fluctuation as well as to know the effect of some plant materials against *H. vigintioctopunctata* beetle under laboratory conditions.

MATERIALS AND METHODS

Study site

The survey was conducted in the campus area of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India during 2022-2023. The experiments on morphological identification, life cycle, morphometrics and management studies of *H. vigintioctopunctata* were conducted at the Entomology laboratory of the Directorate of Research, Regional Research Station (Terai Zone), UBKV, Pundibari, Cooch Behar, West Bengal from November, 2022 to June, 2023.

Collection and identification of *H. vigintioctopunctata*

The eggs, grubs and adults of *H. vigintioctopunctata* were collected randomly by sweep net and hand collection method at weekly interval during the whole survey period. The beetles were collected from different solanaceous crops and some related weeds. Occurrence and diversity of these beetles were assessed by taking observation at weekly interval. The adult beetles were then carried to the laboratory and maintained at room conditions. They were placed in Boro silicate glass vials (6.3 x 2.3 cm diameter) with cotton plugs soaked in ethyl acetate for killing purpose. Larvae collected were reared in petri dishes (9 cm diameter) on respective host plant leaves till adults emerged. The collected specimens were studied in detail and identified using available literature (Chakraborty, Bhowmik, & Biswas, 1996; Tomaszewska & Szawaryn, 2016).

External morphology and genitalia structure

Morphology and morphometric studies

The measurement and morphological features of the different developmental stages of Hadda beetle were observed under laboratory conditions. Measurements of length and width of eggs (n=10), various instars of grub (n=10), pupa (n=10) and adult male and female beetles (n=10) were recorded. The specimens were killed using ethyl acetate in killing bottles, then dried in a hot air oven at 45-50°C for 4-6 hours. The beetles were identified based on the shape of elytra and spot variation with the help of ZEISS Stemi 508 stereo zoom microscope (8:1 zoom) fitted with ZEISS Axiocam 105 color camera and Carl Zeiss Zen 2.5 lite (blue edition) imaging software.

Dissection of male genitalia structures

The male phytophagous coccinellid beetles were separated from females based on the size (generally males are smaller than females) and dissected under a microscope to confirm the sexes. The specimens were positioned dorsally on a dissection slide and elytra removed. After removing elytra, the beetles were turned and kept ventral side upward. The abdomen was detached from the thorax using micro needles under the microscope. With the help of camel hair brush, internal content of the abdomen was removed and kept in 10% KOH solution for 24 hours to dissolve the soft fatty tissue. The abdomen was carefully withdrawn from the KOH solution and placed in a glass hollow dish containing distilled water and added one to two drops of glycerol for genitalia dissection to make the entire abdomen fully transparent and enable for genitalia examination under the stereo zoom microscope and photographed following Roy, Uma Maheswari, Sridevi, & Raghavender (2021).

Biological studies

The field collected gravid female laid eggs in the petri dish (9 cm diameter) and the eggs were reared till adult emergence on the respective host plant leaves under laboratory conditions. Emerged adults were sexed and transferred to new petri dishes (9 cm diameter). In the Laboratory, clean petri dishes were taken and eggs collected from the laboratory reared generation was kept singly on moist filter paper along with tender leaves (*Solanum nigrum*). Twelve such replicates were maintained and observations on incubation period and hatchability were recorded. Daily morning, the food was changed. To prevent desiccation and maintain the freshness, leaves' tip was covered with water-soaked cotton swab. The petri dishes were observed daily till adult emergence. The grubs were examined under microscope for the sign of moulting. In this way, the duration of different instars., 1st, 2nd, 3rd and 4th instar grubs were recorded and also pupal period, total developmental period, adult longevity and fecundity was observed.

Ecological studies

The ecological activity of the Epilachna beetles, *H. vigintioctopunctata* was monitored in a brinjal field during November, 2022 – March, 2023 and afterwards on different solanaceous plants growing within the campus area of Uttar Banga Krishi Viswavidyalaya (UBKV) till June, 2023. In the brinjal field the observations were recorded from five randomly selected plots distributed over the whole field. Again, from each plot data were recorded from ten plants selected at random. During off season the *H. vigintioctopunctata* population was monitored on different solanaceous weed hosts which serve as alternate hosts for the beetles and data were recorded on per plant basis. The meteorological data was obtained from the meteorology unit of UBKV.

Statistical analysis

The analysis of data pertaining to biological parameters and morphometrics were accomplished with the help of range and mean analysis with standard deviation and was performed using Microsoft Excel (2010) software in PC. The seasonal fluctuation

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of the beetle was assessed by considering population counts per plant and the data was plotted in graphical presentations. The grub and adult population was correlated with prevailing weather parameters and correlation coefficients (r) were derived using Microsoft Excel (2010). The significance level of the correlation coefficients at 1% and 5% were judged by comparing the tabulated value with the help of Gomez and Gomez (1984). The influence of weather parameters like average maximum (X_1) and minimum temperature (X_2), maximum (X_3) and minimum relative humidity (X_4) and total rainfall (X_5) on the grub and adult population of *H. vigintioctopunctata* was studied by deriving the linear multiple regression models on a date of observation and average maximum and minimum temperature, maximum and minimum relative humidity and total rainfall during last 7 days from that date of observation. The multiple linear regression model was denoted as follows:

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5$$

Where, Y = Response or dependent variable i.e., pest population

a = Constant or Y intercept which signifies the value of Y when all value of all predictor variables is zero, b_1 - b_5 = Regression coefficients for each predictor variable like X_1 = average maximum temperature, X_2 = average minimum temperature, X_3 = average maximum relative humidity, X_4 = average minimum relative humidity, X_5 = total rainfall.

The 'F' test and student 't' test were applied to test the significance at 5 and 1% level. The Microsoft Excel and OPSTAT statistical package was used for the correlation and regression analysis.

Efficacy of plant extract against *H. vigintioctopunctata*

Extract from whole plant parts viz., flowers, leaves and stem of Biskattali (*Polygonum hydropiper*) and cured Tobacco leaves were tested against the grubs of *H. vigintioctopunctata* under laboratory conditions.

Preparation of botanicals

For this experiment, seven different treatments such as methanolic polygonum extract, aqueous polygonum leaf extract, tobacco decoction, blank methanol solution (to judge the effect of this solvent) and control (water) were used. The Biskattali plants were collected from the campus area of UBKV. The plant material was shade dried for 15-20 days and coarsely grounded in a mixer. The powdered material was extracted with methanol as solvent in Soxhlet apparatus for 3 hours and the resultant semiliquid form of extract was collected in a glass beaker and kept on a water bath at atmospheric pressure to evaporate the excessive amount of solvent. The semi-solid crude extract was collected and stored in refrigerator for further use. From the stock, three concentrations of methanolic extracts of Biskattali (4%, 2% and 1%) were prepared with distilled water (i.e., 4g, 2g and 1g/ 100ml distilled water). For the preparation of aqueous polygonum extract and tobacco decoction, fresh leaf of polygonum and cured tobacco leaf were grounded and soaked (20 g in 100 ml and 25 g in 200 ml distilled water, respectively) overnight. The extract was collected by filtering with the help of filter paper.

Application method

The experimental insect, *H. vigintioctopunctata* was taken from the stock culture being maintained at the Entomology laboratory. Each treatment had 3 replications, so in total 21 petri dishes were maintained for the experiment. In each petridish 10 uniform sized 3rd instar grubs which were starved for 4 hours were released. Along with that set of petridishes 3 more petridishes without grubs were maintained to know the natural loss of leaf weight due to evaporation. The experiment was laid out in a Complete Randomized Design (CRD) with 7 treatments and 3 replications.

Evaluation of leaf protection by the application of different plant extract

Pre-weighed *Solanum nigrum* leaves of uniform size were selected and treated with different treatments and then airdried for 10 minutes. Then these treated leaves were given to the pre-starved grubs for feeding. After 48 hrs. of exposure, the uneaten portions of the leaves were taken out, cleaned and weighed to estimate the quantity of leaves consumed by the grubs.

The percentage of leaf protected by the treatment was estimated by following Lily (1995) as,

$$\frac{A-B}{A} \times 100$$

where,

A = weight of leaf consumed in control and

B = weight of leaf consumed in treatment

RESULTS

Host plants

The Hadda beetle, *H. vigintioctopunctata* were recorded on brinjal, *Solanum melongena* as well as on some solanaceous weed plants like *Solanum nigrum*, *Physalis* spp. and some cucurbits related weeds belonging to the family Cucurbitaceae (Fig. 1a-d). Due to its wide host range, it was recorded throughout the study period.

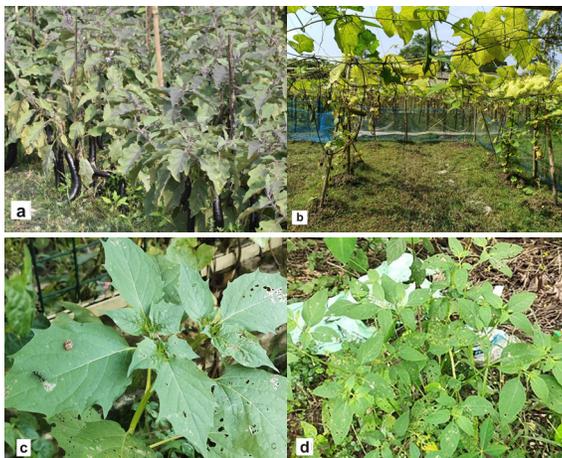


Figure 1. Host plants of *H. vigintioctopunctata*; a) brinjal, b) ridge gourd, c) *Physalis* sp., d) *Solanum nigrum*

External morphology and male genitalia

Eggs: Creamy yellow in colour, slender and elongate-oval in shape with slight round ring like mark on the apex (Fig. 2a).

Grub: Neonate grub was glossy yellow or sometimes light blackish in colour. Second and third instar grubs were shiny yellow in colour with six rows of spine and more or less similar in appearance except body size. The fully matured grubs of *H. vigintioctopunctata* had a black band at the base of the spine. Spines were whitish in colour (Fig. 2b).

Pupa: The pupa of *H. vigintioctopunctata* was creamy white in colour and it had 2 round-shaped black spots in the anterior region and while the posterior margin had white colour spine (Fig. 2c).

Adult: The adult of *H. vigintioctopunctata* was copperish brown in colour with the variation of 7-14 spots on each elytron and it had roundish elytral tip (Fig. 2d).

Male genitalia: The genitalia structure of male *H. vigintioctopunctata* had median lobes with two rows of hairs and a basal knife edge. The base of siphon was long and broad and it had a gentle curve, then the siphonal tube was straight with pointed siphonal tip at the end (Fig. 2e).



Figure 2. External morphology and male genitalia of *H. vigintioctopunctata*; a) egg, b) grub, c) pupa, d) adult, e) male genitalia.

Life cycle and morphometric studies

Eggs were laid as small clusters on the under surface of the leaves. Freshly laid eggs were light yellow in colour but after hatching it turn into white. After hatching of the eggs, the insect passed through four larval instars followed by a pupal stage and finally adult (Fig. 3a-j).

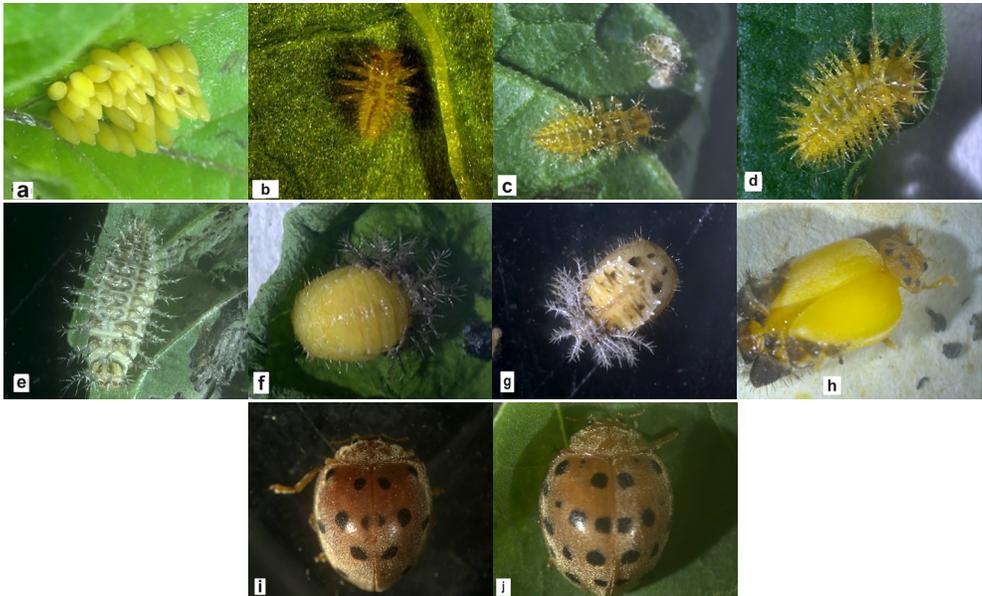


Figure 3. Life cycle of *H. vigintioctopunctata*; a) egg, b) 1st instar larva, c) 2nd instar larva, d) 3rd instar larva, e) 4th instar larva, f) newly formed pupa, g) pupa after 2 days, h) newly emerged adult, i) male adult, j) female adult.

Newly emerged first instar grubs fed gregariously. The larvae scrapped the lower epidermal surface of the leaves and adults fed on upper surface. The incubation period of Hadda beetle ranged from 3 to 5 days with an average of 3.6 ± 0.8 days (Table 1) and the mean length and breadth of egg was 1.33 ± 0.21 and 0.43 ± 0.07 mm, respectively (Table 2). The duration of 1st instar grub was 2-7 days with a mean of 3.8 ± 1.9 days. The average duration of 2nd instar grub was 2-5 days with an average of 3.5 ± 1.1 days. The 3rd instar duration ranged from 2-7 days with a mean of 3.6 ± 1.4 days. The fully grown 4th instar grub required 3-5 days for development with an average of 4.1 ± 0.7 days. The total larval period was 13 – 20 days with an average of 15 ± 2.5 days. The body length and breadth of 1st, 2nd, 3rd and 4th instar larvae was 2.03 ± 0.13 and 1.22 ± 0.15 mm, 2.98 ± 0.11 and 1.47 ± 0.13 mm, 4.07 ± 0.15 and 2.08 ± 0.20 mm & 4.89 ± 0.53 and 2.97 ± 0.43 mm, respectively. The pupal period lasted for 3-5 days with an average of 4.5 ± 1.1 days and the average body length and breadth of pupa was 5.03 ± 0.30 and 3.92 ± 0.16 mm, respectively. The life cycle of *H. vigintioctopunctata* was 20-31 days with a range of 23.1 ± 3.5 days. Generally, the females were bigger in size than the males.

The observed longevity of the male and female was 24-58 and 36-67 days with a mean of 43.3 ± 10.8 and 48.8 ± 9.3 days, respectively (Table 1). The observed mean body length and breadth of male and female was 5.83 ± 0.29 and 4.91 ± 0.39 & 6.86 ± 0.51 and 5.65 ± 0.48 mm, respectively (Table 2). The fecundity of female beetle on *S. nigrum* was found in the range of 142-257 with an average of 220.1 ± 33.0 .

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Table 1. Duration of various life stages of *Henosepilachna vigintioctopunctata*.

Stages	Range (days)	Mean \pm SD (days)
Egg	3 – 5	3.6 \pm 0.8
1st Instar	2 – 7	3.8 \pm 1.9
2nd Instar	2 – 5	3.5 \pm 1.1
3rd Instar	2 – 7	3.6 \pm 1.4
4th Instar	3 – 5	4.1 \pm 0.7
Total larval period	13 - 20	15 \pm 2.5
Pupa	3 – 6	4.5 \pm 1.1
Total Life cycle	20 - 31	23.1 \pm 3.5
Adult longevity		
Male	24 – 58	43.3 \pm 10.8
Female	36 – 67	48.8 \pm 9.3
Fecundity*	142 – 257	220 \pm 33.0

Average of 10 observations.

*Average no. of eggs laid by single female.

Table 2. Morphometrics of different developmental stages of *Henosepilachna vigintioctopunctata*.

Stages	Length (mm) Mean \pm SD	Breadth (mm) Mean \pm SD
Egg	1.33 \pm 0.21	0.43 \pm 0.07
1st Instar	2.03 \pm 0.13	1.22 \pm 0.15
2nd Instar	2.98 \pm 0.11	1.47 \pm 0.13
3rd Instar	4.07 \pm 0.15	2.08 \pm 0.20
4th instar	4.86 \pm 0.53	2.97 \pm 0.43
Pupa	5.10 \pm 0.37	3.92 \pm 0.16
Adult		
Male	5.83 \pm 0.29	4.91 \pm 0.39
Female	6.86 \pm 0.51	5.65 \pm 0.48

Average of 10 observations.

Seasonal incidence

Brinjal

The activity of beetle population was observed from the 3rd week of November, 2022 to the second week of March, 2023 during rabi season in brinjal crop (Fig. 4). The population of grubs and adults of *H. vigintioctopunctata* fluctuated throughout the crop period in the field till maturity. But more population was observed during the vegetative and early fruiting stage of brinjal. Correlation coefficient between grub population and weather parameters revealed that max. temp. ($r=-0.535^*$) showed low level of significant negative association and min. temp. ($r=-0.106$), max. RH($r=0.448$) and min RH ($r=0.176$) had non-significant association. The adult population had highly significant negative correlation with min. temp. ($r=-0.665^{**}$) and non-significant correlation with max. temp. ($r=-0.428$), max. RH ($r=0.453$) and min RH ($r=0.272$) (Table 3).

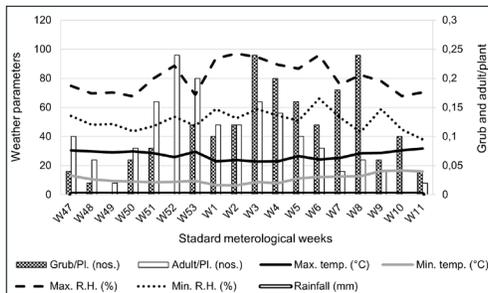


Figure 4. Population of *H. vigintioctopunctata* and mean abiotic factors for preceding week (Brinjal)

Table 3. Correlation coefficients between *H. vigintioctopunctata* in brinjal and weather parameters.

Stages	Max. temp. °C	Min. temp. °C	Max. RH %	Min. RH %	Total Rainfall (mm)
Grub	-0.535*	-0.106	0.448	0.176	-
Adult	-0.428	-0.665**	0.453	0.272	-

*Significant at 5% level of significance.

** Significant at 1% level of significance.

Multiple linear regression: Together, all the abiotic factors were responsible for 29.98% variation in the larval population and 26.07% variation in the adult population of *H. vigintioctopunctata* (Table 4) and the combined effect of all the meteorological parameters was statistically significant at 5% significance level for grubs (F-calculated = 3.93) and adults (F-calculated = 3.39) (Table 5).

Table 4. Multiple linear regression equation of *H. vigintioctopunctata* in brinjal with weather parameters.

Life stages	Regression equation	Coefficient of determination (R2)
Grub	$Y = 1.362 - 0.036X_1 + 0.013X_2 - 0.001X_3 - 0.007X_4$	0.2998
Adult	$Y = -0.393 + 0.012X_1 - 0.015X_2 + 0.003X_3 + 0.002X_4$	0.2607

X1 = Max. temp, X2 = Min. temp, X3 = Maximum RH, X4 = Minimum RH.

*Significant at 5% level of significance.

** Significant at 1% level of significance.

Table 5. Parameters of larval and adult population of *H. vigintioctopunctata* as affected by weather parameters in brinjal.

Variables	Regression Coefficients		Standard Error		t-value		Significance		F-calculated	
	Grub	Adult	Grub	Adult	Grub	Adult	Grub	Adult	Grub	Adult
Max. temp.	-0.036*	0.012	0.013	0.011	-2.869*	1.079	0.011	0.296	3.93*	3.39*
Min. temp.	0.013*	-0.015*	0.006	0.005	2.199*	-2.802*	0.042	0.012		
Maximum RH	-0.001	0.003	0.003	0.003	-0.185	1.086	0.855	0.293		
Minimum RH	-0.007*	0.002	0.003	0.003	-2.298*	0.627	0.035	0.539		
Constant	1.362	0.393	-	-	-	-	-	-		

*Significant at 5% level of significance

Solanaceous weed hosts

The population of *H. vigintioctopunctata* on solanaceous weeds during off season was first observed in the 3rd week of April, 2023. The population of grubs and adults of *H. vigintioctopunctata* fluctuated throughout the study period, but a greater number of beetle population were observed than the main host. The highest adult population was observed on 18th SMW (2 adult/pl), likewise maximum grub population was recorded

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on 24th SMW (0.8 grub/pl) (Fig. 5). The correlation coefficients between the beetle population (both grub and adult) and weather parameters were non-significant. But the beetle population showed positive correlation with maximum ($r=0.274$ & 0.152) and minimum relative humidity ($r=0.174$ & 0.253) and rainfall ($r=0.617$ & 0.137), and negative correlation with minimum temperature ($r= -0.037$ & -0.386). The maximum temperature ($r= 0.072$ & -0.463) acted differently in grub and adult, in case of grub, it showed positive correlation and in adult, it showed negative correlation (Table 6).

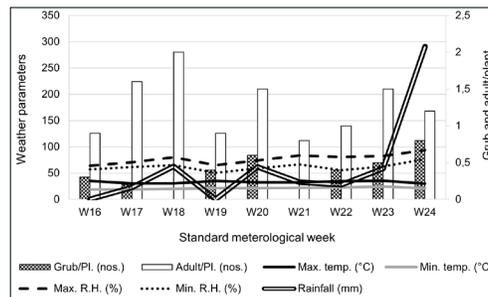


Figure 5. Population of *H. vigintioctopunctata* and mean abiotic factors for preceding week (Solanaeous weeds).

Table 6. Correlation coefficients between *H. vigintioctopunctata* in solanaeous weed and weather parameters.

Stages	Max. temp. °C	Min. temp. °C	Max. RH %	Min. RH %	Total Rainfall (mm)
Grub	0.072NS	-0.037 NS	0.274 NS	0.174 NS	0.617 NS
Adult	-0.463 NS	-0.386 NS	0.152 NS	0.253 NS	0.137 NS

NS – non-significant.

Multiple linear regression: It revealed that together all the abiotic factors were responsible for 73.31% variation in the larval population and 8.49 % variation in the adult population of *H. vigintioctopunctata* (Table 7). From the Table 8, it is evident that beetle population showed non-significant regression coefficients as indicated by the t-test statistics values.

Life stages	Regression equation	Coefficient of determination (R ²)
Grub	$Y = -0.358 + 0.075X_1 - 0.006X_2 - 0.012X_3 - 0.016X_4 + 0.005X_5$ NS	0.7331
Adult	$Y = 2.975 - 0.050X_1 - 0.016X_2 - 0.014X_3 + 0.023X_4 - 0.001X_5$ NS	0.0849

NS – non-significant.

Table 8. Parameters of larval and adult population of *H. vigintioctopunctata* as affected by weather parameters in solanaeous weeds.

Variables	Regression Coefficients		Standard Error		t-value		Significance		F-calculated	
	Grub	Adult	Grub	Adult	Grub	Adult	Grub	Adult	Grub	Adult
Max. temp.	0.075	-0.050	0.064	0.218	1.1750	-0.228	-1.789	0.372	3.5743NS	0.2468NS
Min. temp.	-0.006	-0.016	0.011	0.036	-0.514	-0.425	-0.379	-0.152		
Maximum RH	-0.012	-0.014	0.019	0.064	-0.646	-0.219	-0.702	0.398		
Minimum RH	-0.016	0.023	0.029	0.099	-0.548	0.236	-0.463	0.350		
Rainfall	0.005	-0.001	0.001	0.004	3.893	-0.238	-3.308	0.345		
Constant	-0.358	2.975	-	-	-	-	-	-		

NS – non-significant

Efficacy of plant extracts against *H. vigintioctopunctata*

The present study revealed that there was variation in leaf protection by different treatments (Fig. 6). But among the treatments, 4 % methanolic Polygonum extract gave the highest mean percentage of leaf protection (68.13%) followed by 2% methanolic Polygonum extract (57.08%) and 1% extract (48.04%). The aqueous Polygonum extract resulted in 37% leaf protection over control, which was higher than the Tobacco decoction (18.56%). The mean percent of leaf protection by a blanket solvent (methanol) application was 13.6%.

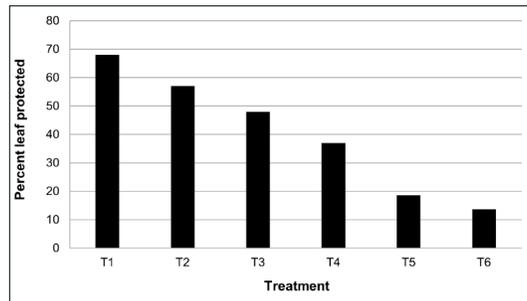


Figure 6. Percent leaf protected by different treatments.

DISCUSSION

The species *Henosepilachna vigintioctopunctata* under the tribe Epilachnini, is the most serious and widespread pest of Solanaceous vegetables and also occur on some related weed plants. Poorani *et al.* (2021) reported that, *H. vigintioctopunctata* and *H. septima* were the most widely distributed and economically important plant feeding coccinellids in India. Similarly, many works have been conducted on the host plants of phytophagous coccinellids. Naz, Inayatullah, Rafi, Ashfaque, & Ali (2012) recorded the host plants of *H. vigintioctopunctata* such as *Solanum melongena*, *S. nigrum*, *S. surretanses*, *Withania somnifera*, *Datura*, *Lycopersicum esculantum* and *Physalis* spp. In 1988, Anand, Gupta, & Ghai reported *H. vigintioctopunctata* from various crops such as *S. melongena*, *S. nigrum*, *S. surretanses*, *S. tuberosum*, *S. carolinense*, *S. xanthocarpum*, *S. indicum*, *S. khasianum*, *S. pubescent*, *S. evieulaare*, *S. torvum*, *S. megacarpum*, *Datura fastuosa*, *D. stramonium*, *Luffa cylendrica*, *W. somnifera*, *Momordica charantia*, *L. esculantum* and *P. maxima*, pumpkin, gourds, zucchini, mamours and cucurbits, *Cucurbita moschata*, cotton, melon, rock melons, cucumber, squash, *Luffa aegytiaca*, *Lagenaria* sp., *Vigna unguiculata* (cowpea), *Arachis hypogaea* (groundnut), alfalfa, *Phaseolus mungo*, *Hibiscus esculentum*, *Musa* sp. (banana); *Raphanus sativus* and *Zea mays* from India. Due to its polyphagous nature, it had higher chances of survival rate.

Generally, species of phytophagous coccinellid beetles are distinguished based on the elytral spots, colour and shape of the elytra. But elytral spots cannot be considered as full proof confirmation for species identification as the spots tend to vary as found

by earlier workers like Dharmaretham (2002). Elytral spots variation is influenced by genetic as well as environmental factors like ambient temperature. For further confirmation genitalia dissection of male beetles has to be done. Naz et al. (2012) described the Epilachna beetle, *Epilachna vigintioctopunctata* based on the external morphology and genital structure. The collected specimens were ground colour pale brown or reddish brown, elytral apex angled. Spots on the elytra varied between 12 and 28 but mostly with 26 spots and 7 pronotal spots. Saeed et al. (2016) collected two species *H. vigintioctopunctata* and *H. septima* during the study of morphological characteristics of ladybird beetles in Pakistan. The body shape of *H. vigintioctopunctata* was rounded with elytral apex also rounded, but the elytral spots varied, whereas, in *H. septima* the elytral apex was angular. Elytral spot pattern was found variable. Dharmaretham (2002) distinguished two species viz., *H. vigintioctopunctata* and *H. septima* based on genital structure. In the male genitalia of *H. septima*, the apex of the siphon was sharply narrowed on one side and pointed. In *H. vigintioctopunctata*, the median lobe of male genitalia was with basal knife edge and two rows of hairs. The siphon was gently curved near the base, then straight, ending in a curved point.

The biology of *H. vigintioctopunctata* has earlier been studied by several authors, Qamar, Masarrat, & Sharma (2009) and Kaur & Mavi (2005) recorded the incubation period of Epilachna beetle as 4 days and 5.20 ± 0.87 days on brinjal. Our results were confirmed with the findings reported by Kaur & Mavi (2005), the duration of first, second, third, and fourth instar larvae were 3–4, 2–4, 2–4, and 2–5 days, respectively with an average of 2.20 ± 0.40 , 3.60 ± 0.66 , 5.70 ± 0.46 , and 4.10 ± 0.54 days on brinjal. In 2009, Hossain, Khan, Haque, Mannan, & Dash reported that the duration of 1st, 2nd, 3rd and 4th grub instars were 2.25 ± 0.13 , 3.25 ± 0.08 , 2.55 ± 0.15 and 3.25 ± 0.12 days, respectively. Tayde & Simon (2013), recorded the pupal period of Hadda beetle to be in the range of 3- 5 days with an average of 4.4 ± 0.89 days and the average longevity of male and female was 51.4 and 64.8 days, respectively. The life cycle of Hadda beetle was similar with the findings observed by Chowdhuri (1965), Ghosh & Senapati (2001), *H. vigintioctopunctata* required 22-30 days for its development and also, they stated that the life cycle duration might be varied depending upon the species of host plants on which it feeds and prevailing climatic conditions.

According to Kaur & Mavi (2005), newly laid eggs length and breadth was 1.09 ± 0.04 mm and 0.32 ± 0.03 mm, respectively. Verma & Anandhi (2008) reported that an egg's average length and breadth was 1.13 ± 0.10 mm and 0.41 ± 0.07 mm. Tayde & Simon (2013), recorded the average length and breadth of 1st, 2nd, 3rd and 4th instars were 2.09 ± 0.02 mm and 1.12 ± 0.26 mm, 2.99 ± 0.32 mm and 1.43 ± 0.16 mm, 4.16 ± 0.17 and 1.92 ± 0.31 & 6.18 ± 0.37 and 3.02 ± 0.34 mm, respectively. Our findings draw support from the results earlier obtained by Tayde & Simon (2013), who reported the average pupal length and breadth as 6.15 ± 0.24 mm and 3.73 ± 0.38 mm, respectively. The average body length of male was 6.08 ± 0.25 mm and breadth was 4.71 ± 0.43 mm. Whereas, in female length was 7.13 ± 0.49 mm and breadth was 5.41 ± 0.58 mm, respectively.

The present study results revealed that the population dynamics of *H. vigintioctopunctata* was influenced by environmental conditions either significantly or non-significantly. During Rabi season, the beetle population was started increasing from January month. Similarly, Haseeb, Qamar, & Sharma (2009) noted the initial incidence of the *H. vigintioctopunctata* on third week of January, 2009 in Aligarh, Uttar Pradesh. In 1999, Raghurajan and Veeravel reported that the *H. vigintioctopunctata* population was highest in February and March in brinjal. In Solanaceous weeds, the highest beetle population was observed on 18th, 20th and 24th standard meteorological week. Similarly, Qamar & Haseeb (2019) observed the peak grub and adult beetle population during 20th and 23rd SMW on tomato. They also concluded that *Henosepilachna* spp. can adopt to a vast range of temperature (27 to 35°C), relative humidity (24 to 83%) and rainfall (0.80 to 70 mm). But the adoption of these climatic conditions by *Henosepilachna* spp. depends on the crop season and crop type, which enable the beetle to thrive well.

Different plant parts of *Polygonum hydropiper* was extracted with a solvent and bioassay of various concentration of methanolic polygonum extract, polygonum aqueous and Tobacco decoction were tested against 3rd instar grub of *H. vigintioctopunctata*. These two are most commonly and easily available plant materials for the Terai region of West Bengal (India). Polygonum has been used as the main plant extract i.e., different treatments and tobacco was used as a botanical check, well known for its efficacy under this zone. In this study, the antifeedant activity of different treatments against *H. vigintioctopunctata* was estimated based on their leaves consumption rate. The result revealed that 4% methanolic polygonum extract showed better result followed by other treatments. Similarly, Podder, Uddin, & Adnan (2013), assessed the effectiveness of four botanicals, Neem Oil, Mehagony Oil, Bishkatali Leaf Extract, Pithraj seed extract and one insecticide Diazinon 60 EC against Epilachna beetle. Results revealed that, the aqueous extract of all the four botanicals were effective in beetle population reduction. Among the botanicals neem oil was found to be more toxic ranging next to Diazinon 60 EC. The Biskattali leaf extract showed better result compared to control. The past couple of decades have seen encouraging progress in the control of Epilachna beetles that infest several economic crops. While *H. vigintioctopunctata* infesting brinjal, Sreedevi, Chitra, & Rao (1993) and Mehta, Vaidya, & Kashyap (1995) observed the effectiveness of some plant extracts against *H. vigintioctopunctata* infesting brinjal. Under laboratory condition, Hussain (1995) observed some toxic effect of Bishkattali (*P. hydropiper*) leaf powder and extract on the *Tribolium castaneum* larvae.

CONCLUSION

The plant feeding coccinellid, *H. vigintioctopunctata* persists in the agroecosystem of sub-Himalayan eastern India almost throughout the year by changing their feeding habitat between cultivated crops and wild host plants. The duration of various life stages of *H. vigintioctopunctata* on the *S. nigrum* is quite comparable with other

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cultivated crops. So, this plant can be used as a potential trap crop for the management of this pest. The plant *Polygonum hydropiper* which the farmers can access very easily in and around their farm lands can be utilized successfully coinciding initial pest infestation to check the population attaining the economically damaging level.

ACKNOWLEDGEMENT

We are thankful to the Department of Agricultural Entomology, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal for providing the facilities.

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New Records of Iranian Grass Gall Midges (Diptera: Cecidomyiidae)

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ABSTRACT

The diversity of plant species belonging to the Poaceae family in Iran is very rich with about 500 known species. Many species of gall midges (Diptera: Cecidomyiidae) have a feeding relationship with plants of the Poaceae family. Despite the great species richness of Poaceae in Iran and the association between Cecidomyiidae/Poaceae, only 6 species of gall midges have been collected and identified from the plants of the Poaceae in Iran. In the present investigation related to the gall midge fauna of Iran, 3 genera namely, *Calamomyia* Gagné, 1969, *Epicalamus* Sylvén, 1998 and *Mayetiola* Kieffer, 1896 and 13 species namely, *C. echinochloa* Felt, 1916, *Contarinia festucae* Jones, 1940, *C. floricola* (Oettingen, 1927), *C. lolii* Metcalfe, 1933, *Dasineura alopecuri* (Reuter, 1895), *E. phalaridis* Sylvén, 1998, *Lasioptera arundinis* Schiner, 1854, *L. calamagrostidis* Rübssaamen, 1893, *L. donacis* Coutin, 2001, *Mayetiola poae* (Bosc, 1817), *Stenodiplosis sorghicola* (Coquillett, 1899), *S. geniculati* (Reuter, 1895) and *S. panici* Plotnikov 1926 are reported for the first time from the country. The adult specimens were obtained by rearing from their larvae on 13 genera and 13 species of Poaceae. The genus *Calamomyia* Gagné 1969, which is distributed in the Nearctic region, is reported for the first time from the Palearctic region.

Keywords: fauna, Cecidomyiinae, Poaceae, host plant, Iran.

INTRODUCTION

Midges of the family Cecidomyiidae (Diptera, Nematocera) presently includes 6651 known species and 832 genera with a worldwide distribution. They form 6 subfamilies, including Catotrichinae, Lestremiinae, Micromyinae, Winnertziinae, Porricondylinae and Cecidomyiinae (Gagné & Jaschhof, 2021). Excluding the Cecidomyiinae, which are primarily herbivores with a number of aphidophagous species, the other subfamilies comprise about one-fourth of the known species, and are all fungivores. Many Cecidomyiidae induce galls and therefore are commonly known as gall midges. Many species of this subfamily have economic importance as pests of cultivated plants or biological control agents of weeds and as predator of harmful insects such as aphids (Gagné & Jaschhof, 2021; Kolesik & Gagné, 2020).

The plant species belonging to Poaceae Barnhart (Poales) is the second most important family in terms of the number of genera with about 130 genera and the third most important family in Iran in terms of the number of species with about 500 species (Ghahremaninejad & Nejad Falatoury, 2016).

In the catalogue provided by Gagné & Jaschhof (2021), about 144 species of gall midge species of the world are associated with Poaceae. About 55% of them are from the genus *Mayetiola* Kieffer, 1896, *Orseolia* Kieffer & Massalongo, 1902, and *Contarinia* Rondani, 1860, families.

Some of them are among the most important pests of crops such as wheat: the orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin, 1857), the yellow wheat blossom midge, *Contarinia tritici* (Kirby, 1798) and the saddle gall midge, *Haplodiplosis marginata* (Roser, 1840) (Chavalle, Buhl, San Martin y Gomez, & De Proft, 2018). and rice: *Orseolia oryzae* (Wood-Mason, 1889) (Jagadeeshakumar, Chakravarthy, Doddabasappa, & Basavara, 2009).

Despite the great species richness of Poaceae family in Iran, the collection and identification of gall midges associated with Poaceae are rare. To date, only six genera and six species have been identified from Iran. The aim of this study is to search and identify more species of gall midges' fauna associated with Poaceae in Iran/west Azerbaijan province.

MATERIAL AND METHOD

First of all, a list of gall midge's host plants from the Poaceae family was prepared using the Gagné & Jaschhof (2021) and Skuhravá & Skuhravý (2021).

After identifying the grasses that were host plant for gall midges in the area, plant materials suspected of gall midge larvae were collected during the year 2020-2022 from ten districts of west Azerbaijan province, Iran (Fig. 1). Collected plant materials, were transported to the laboratory in plastic bags. Collection dates of plant materials were recorded. After transferring to laboratory, plant materials were kept separately according to species 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

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a height of 2 cm so that the species that pupate in the soil have a place for pupation. All boxes were checked daily for collection of emerging gall midge adults for 25-30 days. Emerged adults were put in vials with 75% ethanol and kept for identification. Some studied adult specimens were mounted on permanent microscope slides using Canada balsam as medium according to the method described by Jaschhof & Jaschhof (2009).

The specimens were identified using the keys written by Skuhravá (1997). Gagné & Jaschhof (2021) were used to obtain the known geographic distribution of each species.

All voucher specimens, both on slides and in ethanol, are deposited in the second author's collection at the Břitovská 1227/9, CZ-140 00 Praha 4, Czech Republic. The species are listed in alphabetical order by the genus.



Figure 1. *Sampled area and the distribution map of identified gall midge's species. (Google Earth, 2023)
 1) *Calamomyia echinochloa* and *Stenodiplosis panici*, 2) *Contarinia lolii*, 3) *Lasioptera arundinis* and *Mayetiola poae*, 4) *Contarinia floricola*, *Dasineura alopecuri* and *Lasioptera donacis*, 5) *Contarinia festuca* and *Stenodiplosis sorghicola*, 6) *Epicalamus phalaridis*, 7) *Contarinia lolii*, 8) *Lasioptera donacis*, 9) *Stenodiplosis geniculati*, 10) *E. phalaridis* and *Lasioptera calamagrostidis*.

* The areas shown on the map are approximate.

RESULTS AND DISCUSSION

Keeping the plant materials under the appropriate laboratory conditions led to the gradual emergence of adult gall midges. All the examined materials of adult gall midges appeared in the rearing cages within 25 to 30 days after keeping the plant material in the laboratory. The adults belonged to seven genera and thirteen species as follow:

Calamomyia Gagné, 1969**Calamomyia echinochloa Gagné, 1969***Lasioptera echinochloa* Felt, 1916**Distribution:** USA, South Dakota, host plant: *Echinochloa crus-galli* (L.) (Poaceae).**Material examined:** Khoy (vicinity of Pirkandī village, sugar beet field), 38° 43' 30" N, 45° 06' 36" E, 1000 m asl., plant materials collection date (pmcd): 13 Aug. 2020, ♀♂**Host plant:** *E. crus-galli* L.**Remark:** Genus *Calamomyia* Gagné 1969 comprises 13 species. All known species of *Calamomyia* were recorded from USA (Felt, 1916). Therefore, this is the first report of genus *Calamomyia* from the Palaearctic region. With the record of *C. echinochloa* from Iran, the distribution of *Calamomyia* spreads to the Palearctic region.**Contarinia Rondani, 1860****Contarinia festucae Jones, 1940****Distribution:** Bulgaria, Czech Republic, Germany, Poland, Slovakia, Sweden, United Kingdom; Host plant: *Festuca pratensis* Huds., *F. rubra* L. (Poaceae).**Material examined:** Urmia, vicinity of Band village, 37°27'46" N, 44°55'33" E, 1480 m asl. margins of spruce grove, pmcd: 2 July 2020, ♀♂**Host plant:** *Festuca ovina*. Yellow coloured larvae live in inflorescences of *Festuca* spp. without any external deformation.**Remark:** First record from the western parts of Palearctic region.**Contarinia floricola (Oettingen, 1927)***Contarinia poae* Barnes 1946**Distribution:** Czech Republic, Germany, Poland, Slovakia, Sweden, United Kingdom. Host plants: *Poa* sp.; *P. pratensis* L., *P. trivialis* L. (Poaceae).**Material examined:** Urmia, Sero, 37°43'18" N, 44°42'43" E, 1531 m asl., sugar beet field margins, pmcd: 4 Aug. 2020 and 4 May 2021, ♀♂**Host plant:** *Poa trivialis*. Several yellow coloured larvae live in seeds of *Poa* spp. One generation develops per year.**Remark:** First record from the western parts of Palearctic region**Contarinia lolii Metcalfe, 1933****Distribution:** Poland, United Kingdom; host plant: *Lolium pratense*.**Material examined:** Urmia, Sir Mountain, vicinity of Kelisā Kandī village 37°29'08" N, 45°01'31" E, 1638 m asl., pmcd: 4 Sept. 2020, ♀♂; Urmia, Ghoshchi pass, 38°01'36"N, 44°57'46"E, 1638 m asl., pmcd: 7 Aug. 2020, ♀♂**Host plant:** *Lolium perenne*. Several yellow-coloured larvae develop in the inflorescences of *L. perenne*.

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Remark: First record from the western parts of Palaearctic region. The genus *Contarinia* has 318 species that are distributed in different regions of the world. Many of them live gregariously in the flower heads and leaf rolls of host plants. Till now three species of genus *Contarinia* have been reported from Iran, including *C. desertorum* Marikovskij, 1961 [host plant: *Alhagi maurorum* Medik. (Fabaceae)], *C. tritici* (Kirby, 1798) [host plant: *Triticum vulgare* L. (Poaceae)] and *Contarinia* sp.1 [host plant: *Echinophora orientalis* Hedge & Lamond (Apiaceae)] (Karimpour & Skuhravá, 2012; Farahbakhsh, 1961). *Alhagi maurorum* Medik.

Dasineura Rondani, 1840

***Dasineura alopecuri* (Reuter, 1895)**

Oligotrophus alopecuri Reuter, 1895: 3

Dasyneura agropyronis Barnes, 1927: 214

Distribution: Bulgaria, Czech Republic, Denmark, Finland, Germany, Poland, Sweden, United Kingdom. Immigrant to north America (Canada (Ontario, New Brunswick) and New Zealand. Host plant: *Alopecurus pratensis* L.; *Agropyron repens* (L.) (Poaceae).

Material examined: Urmia, Sero, 37°43'18" N, 44°42'43" E, 1531 m asl, sugar beet field margins., pmcd: 19 July 2020, ♀♂

Host plant: *Agropyron repens*. Orange to brick-red larvae live solitary in florets of *A. pratensis* and *A. repens*.

Remark: First record from the western parts of Palaearctic region. Ten species of the genus *Dasineura* Rondani, 1840 have been reported from Iran (Skuhravá Skuhravá, Karimpour, Sadeghi, Gol & Joghataei, 2014; Karimpour, Skuhravá. Lotfalizadeh & Razmi, 2022). The present species stands as eleventh.

Epicalamus Sylvén, 1998

***Epicalamus phalaridis* Sylvén, 1998**

Distribution: Sweden, Host plant: *Phalaris arundinacea* L. (Poaceae).

Material examined: Naghadeh, near the Solduz wetland, 37°02'29" N, 45°36'53" E, 1280 m asl, pmcd: 28 April 2021, ♀♂; Urmia, vicinity of Shahar Chate dam, 37°27'38"N, 44°54'57"E, 1600 m asl., pmcd: 22 May 2021, ♀♂; Urmia, vicinity of Band village, pmcd: 2 July 2020, ♀♂

Host plant: *Phalaris arundinacea*. Up to 4 mm large, and orange colour larvae live gregariously within leaf sheath.

Remark: Iran is the second country where this species is reported. This genus comprises one species is known as a severe pest of *P. arundinacea* in Sweden. Larvae feed beneath the leaf sheaths and cause severe damage (Hellkvist, Finell & Landström, 2003).

Lasioptera* Meigen, 1818**Lasioptera arundinis* Schiner, 1854**

Distribution: Widespread in Europe including, Austria, Belgium, Bulgaria, Crete, Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, Malta, Netherlands, Poland, Serbia, Slovakia, Switzerland and United Kingdom; Host plant: *P. australis* (Skuhrová, M. & Skuhrový, 2021).

Material examined: Urmia, Urmia University campus, 37°39'14" N, 44°58'35" E, 1360 m asl., pmcd: 8 May 2022, ♀♂

Host plant: *Phragmites australis* (Cav.) Trin. The whitish larvae of *L. arundinis* live gregariously in the swollen lateral shoots of common reed, *Phragmites australis* in the mutualistic relationship with the fungus *Radulidium subulatum* (de Hoog). The fungus allows the larva to penetrate into the stem and to have access to the vascular tissues. The fungus protects the larva against parasites and allows an easy exit of the imago. The adult gall midge and the first larval stage have adapted structures to carry the fungus (Rohfritsch, 1992; Arzanlou et al, 2007). The occurrence of this fungus has been reported in Iran, including around Urmia Lake (Salimi, Alizadeh, Mirzadi Gohari, & Javan-Nikkhah, 2019).

Only one generation develops per year (univoltine). Hibernation and pupation takes place in the gall.

Remark: To date, three species of gall midges namely, *Asynapta phragmitis* (Giraud, 1863), *Lasioptera flexuosa* (Winnertz, 1853) and *Giraudiella inclusa* (Frauenfeld, 1862) have already been reported from common reed in Iran, west Azerbaijan province, Urmia (Skuhrová et al, 2014; Karimpour et al, 2022). With report of *L. arundinis* in this paper the number of gall midge species that are associated with common reed in Iran (west Azerbaijan province) reaches to four species.

First record from the western parts of Palearctic region.

***Lasioptera calamagrostidis* Rübsaamen, 1893**

Lasioptera graminicola (Kieffer, 1898)

Distribution: Czech Republic, Denmark, France, Germany, Hungary, Latvia, Netherlands, Poland, Slovakia, Ukraine, United Kingdom; Host plant: *Calamagrostis epigeios*, also other grasses (Poaceae).

Material examined: Naghadeh, vicinity of Solduz wetland, 37°02'29" N, 45°36'53" E, 1280 m asl., pmcd: 21 July 2020 and 7 Sept.2021, ♀♂

Host plant: *Calamagrostis epigeios* (L.) Roth. Larvae live under the leaf sheath of *C. epigeios*.

Remark: First record from the western parts of Palearctic region.

***Lasioptera donacis* Coutin, 2001**

Lasioptera donacis Coutin & Faivre-Amiot, 1981

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Distribution: Bulgaria, Egypt, France, Greece, Italy, Malta; Host plant: *Arundo donax* L.

Material examined: Urmia, Ziveh, 37°15'43" N, 44°53'51" E, 1470 m asl, pmcd: 27 July 2020, ♀♂; Urmia, Sero, 37°43'18" N, 44°42'43" E, 1531 m asl., pmcd: 4 Aug 2020, ♀♂

Host plant: *Arundo donax* L. Larval infestation of *A. donax* stems by *L. donacis* is always associated with the presence of a saprophytic fungus, *Arthrinium arundinis* Corda (Xylariales: Apiosporaceae), which is believed to provide a trophic resource for the larvae in the reed leaf (Goolsby et al, 2013; Thomas & Goolsby, 2015; Bon et al, 2018).

The larvae live in the corridors created by a stem-mining agromyzid fly, *Cerodontha* sp. (Diptera) inside the stems and feed on the mycelium of the fungi *Arthrinium arundinis* (Corda) (Coutin & Faivre-Amiot, 1981).

Remark: First Asian record of the species. Till now, three species of *Lasioptera* namely, *L. carophila* Löw, 1874, *L. umbelliferarum* Kieffer, 1909 and *L. flexuosa* (Winnertz, 1853) were reported from Iran (Skuhrová et al, 2014). With this report, their number goes to four species.

***Mayetiola* Kieffer, 1896**

***Mayetiola poae* (Bosc, 1817)**

Mayetiola graminis (Fourcroy, 1785)

Cecidomyia graminis (Brischke, 1869)

Distribution: Austria, Belgium, Bulgaria, Czech Republic, France, Germany, Hungary, Italy, Latvia, Lithuania, Luxemburg, Netherlands, Poland, Romania, Switzerland, United Kingdom, Yugoslavia; Host plant: *Poa nemoralis* L.

Material examined: Urmia University Campus, 37°39'14" N, 44°58'35" E, 1360 m asl., pmcd: 4 May 2021, ♀♂

Host plant: *Poa bulbosa* L. White larvae of *M. poae* cause malformations on the stems of *P. nemoralis*. The species has one generation per year. Hibernation occurs as fully developed larvae in the brown puparium in the gall and they pupate in the gall in spring.

Remark: This is the first Iranian record of genus *Mayetiola* and Asian record of the species.

***Stenodiplosis* Reuter, 1895**

***Stenodiplosis sorghicola* (Coquillett, 1899)**

Contarinia sorghicola Coquillett, 1899

Contarinia andropoginis Felt, 1921

Contarinia saltata Felt, 1919

Contarinia palposa Blanchard, 1958

Contarinia andropogonis Harris, 1964

Distribution: *Stenodiplosis sorghicola* occurs in Africa, India (Tamil Nadu), southeastern China, Japan (Honshu, Kyushu) and the Philippine (Luzon). It is non-native in southern USA, Mexico, Dominican Republic and Argentina. Although *S. sorghicola* originated in Africa, it has recently spread in various countries of the world wherever sorghum is grown. Albania, Greece, Italy and southern France are countries in Europe where this species is found (Skuhrava & Skuharvy, 2021).

Material examined: Urmia, vicinity of Band village, 37°27'46" N, 44°55'33" E, 1480 m asl., pmcd: 7 Aug 2020, ♀♂

Host plant: *Sorghum halepense* (L.) Pers. The larvae of *S. sorghicola* feed on the newly formed ovaries of growing sorghum seeds inside the inflorescences which leads to ear sterility. It is agricultural pest on *Sorghum bicolor* and reproduce several generations per year (multivoltine).

Stenodiplosis geniculati (Reuter, 1895)

Distribution: Bulgaria, UK, Sweden, Finland, Poland; immigr.: Canada (Ontario, New Brunswick), New Zealand USA (South Dakota), New Zealand. *Alopecurus geniculatus*; *A. arundinaceus*, *A. pratensis* (Poaceae).

Material examined: Urmia, Mahabad road, vicinity of Arablū village, 37°24'40"N, 45°14'53"E, 1278 m asl., ten-year-old vineyard, pmcd: 16 July 2021, ♀♂

Host plant: *Alopecurus myosuroides* Huds.

Stenodiplosis panici (Plotnikov, 1926)

Distribution: Yugoslavia, Ukraine, Russia (Europe), Kazakhstan, Uzbekistan, China (widespread). *Panicum miliaceum*, *E. crus-galli* (Poaceae).

Material examined: Khoy, vicinity of Pīrkandī village, sugar beet field., 38° 43' 30" N, 45° 06' 36" E, 1000 m asl., pmcd: 18 Aug., 2020, ♀♂

Host plant: *Echinochloa crus-galli* (L.). Orange colored larvae live gregariously in the flowers of host plants.

Remark: Genus *Stenodiplosis* comprises 14 species with a worldwide distribution. All of which live in the inflorescences of Poaceae family (Gagné & Jaschhof, 2021). Previously, the species *S. bromicola* (Marikovskii & Agafonova, 1961) has been reported from Iran by rearing on inflorescences of *Setaria glauca* (L.) (Poaceae) (Karimpour et al, 2022). With the introduction of these three species, the number of species of this genus in Iran increases to four species.

CONCLUSION

Despite the high species richness of Poaceae in Iran and its western neighboring countries, the study of gall midges in these regions is scanty. Of which, only six genera and six species namely, *Contarinia tritici* (Kirby, 1798), host plant: *Triticum*

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vulgare L., (Farahbakhsh, 1961); *Asynapta phragmitis* (Giraud, 1863), host plant: *Pharagmites australis* L. (Skuhrová et al, 2014); *Giraudiella inclusa* (Frauenfeld, 1862), *Lasioptera flexuosa* (Winnertz, 1853), host plant: *P. australis* and *Stenodiplosis bromicola* (Marikovskii & Agafonova, 1961), host plant: *Setaria glauca* (L.) (Karimpour, Skuhrová, Lotfalizadeh & Razmi, 2022) and *Orseolia cynodontis* Kieffer & Massalongo, 1902, host plant: *Cynodon dactylon* (L.) Persoon (Karimpour & Skuhrová, 2022) were reported from Iran.

In this study, 13 species of gall midges associated with the Poaceae family were collected and identified. With the identification of *C. echinochloa* from Iran, its geographical distribution areas was expanded from the Nearctic region to the Palaearctic biogeographical region. Also, 10 species gall midges were reported for the first time from Asia (west of the Palaearctic region), which indicates the presence and distribution of the above species in this geographical region.

Including 3 genera and 13 species found in this survey, the number of gall midge genera and species related to Poaceae in Iran increases to 9 and 19, respectively. Furthermore, by introducing these genus and species, the number of known gall midge fauna of Iran reaches to 41 genera and 80 species

The gall midge's fauna of Türkiye and Armenia are relatively well studied (Skuhrová, Bayram, Cam, Tezcan, & Can, 2005; Mirumian, 2011; Mirumian & Skuhrová, 2022). In Turkish gall midge fauna two species namely, *Mayetiola destructor* (Say, 1817) and *Mayetiola hordei* Kieffer, 1909 were mentioned. In the fauna of Armenia, *Mayetiola destructor* (Say, 1817) and *Contarinia tritici* (Kirby, 1798), host plant: *Triticum vulgare* Vill. have been reported by Mirumian, (2011). In Iraqi fauna, one species namely, *G. inclusa* and in the Azerbaijan fauna, no species is mentioned (Gagné & Jaschhof, 2021).

Examining the results of published research shows that grass-feeding gall midge fauna is relatively unexplored in Iran, like it is in other areas such as Türkiye, Iraq and Armenia. The results of current study revealed that more surveys are required to document the gall midge communities. Certainly, many genera and species of gall midges have remained unknown due to lack of investigation in these areas.

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New Records of the Moroccan Soldier Flies (Diptera: Stratiomyidae) with the Description of the Male of *Oxycera flava* (Lindner, 1938)

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ABSTRACT

New data about Moroccan soldier flies (Stratiomyidae) are presented in the present study that discusses 15 species. Six species are recorded as new to the fauna of Morocco, four of which, *Oxycera analis* Wiedemann in Meigen, 1822, *O. flava* (Lindner, 1938), *O. marginata* Loew, 18959, and *Chorisops tibialis* (Meigen, 1820) are new records for North Africa. *Oxycera morrisii* (Curtis, 1833) is recorded for the first time in Morocco. *Hermetia illucens* (Linnaeus, 1758) was recently reported based on a personal communication without details but is now also formally added. It adds a new subfamily to the Moroccan fauna, the Hermetiinae. Notes on taxonomy, distribution and biology of the species are added. The male of *O. flava* is described and illustrated.

Keywords: New records, Morocco, Rif Mountains, Palaearctic Region.

Yimlahi, D., Belqat, B., Üstüner, T., & Beuk, P.L.TH. (2024). New records of the Moroccan soldier flies (Diptera: Stratiomyidae) with the description of the male of *Oxycera flava* (Lindner, 1938). *Journal of the Entomological Research Society*, 26(1), 93-112.

Received: November 10, 2023

Accepted: March 20, 2024

INTRODUCTION

At present, the world fauna of Stratiomyidae consists of approximately 2700 recognized and described species belonging to approximately 400 genera (Woodley, 2001). Contributions to the knowledge of stratiomyids of Morocco are very fragmented and remain patchy; the first records were by Becker and Stein (1913) and further studies were those of Séguy (1930) and Lindner (1936-1938) who recorded species in their studies of the Diptera of Morocco or of the Palaearctic stratiomyids, respectively.

One single comprehensive study on the Moroccan stratiomyid fauna was published to summarize the knowledge in the first checklist of the soldier flies of Morocco (Yimlahi et al, 2017). It counted 33 species of which twelve were new additions to the list of Moroccan Stratiomyidae. Kettani and Woodley (2022) uncovered a few other records from the literature and set the total of Moroccan species at 40. With the present publication, another six species are added bringing the total to 46. However, the inventory of the Moroccan fauna is evidently not complete and new investigations are needed.

MATERIAL AND METHODS

The study is based on a total of 455 specimens (293 larvae, 79 males and 83 females) collected from 18 sampling sites in Morocco between 2014 and 2020. Adults were collected by sweep netting, trapped in a malaise tent or reared. The rearing of larvae and nymphs in the laboratory from substrates collected in the field was conducted using the technique employed by Afzan and Belqat (2016) which proved successful in obtaining adult specimens from larvae and nymphs of various Dipteran families, including Stratiomyidae, which are typically found buried in substrates. These substrates primarily consisted of silt, mosses, and submerged or partially submerged vegetation, collected from riverbeds, banks, and aquatic environments, and transported back to the laboratory in large containers. The experimental rearing protocol involved using basins and mesh screens, onto which the substrate was spread, and then covered with additional mesh to contain the emerging adults. We have also reared adults of *Hermetia*, both in the wild (Fig. 1) and in the laboratory, from larvae found in the litter (straw and plant food remains) of rabbits raised on a small farm. The specimens examined were collected in Chefchaouen, Ifrane, Khénifra, Tanger-Assilah, Larache, Tétouan, M'diq-Fnideq and Sidi Kacem Provinces. The specimens were sampled by the first and the second authors. All specimens are deposited in the insect collection of the Laboratory Ecology, Systematics, Conservation of Biodiversity in the Department of Biology, Faculty of Sciences of Tétouan, Abdelmalek Essaâdi University.

The list of sampling sites, with their coordinates and elevations, is presented in Table 1. Photographs of sampling localities showing Moroccan habitats of Stratiomyids are given.

The identifications were made using Séguy (1930), Rozkošný (1982, 1983) and Mason (2013). The nomenclature and the list of the species known from Morocco follow Woodley (2001).

New Records of the Moroccan Soldier Flies (Diptera: Stratiomyidae)

Table 1. Sampling sites (in alphabetical order) with province and locality names, elevation (meters), and geographical coordinates (latitude/longitude).

Site	Province, locality	Geographical coordinates	Elevation (m)
Rif Mountains			
Aazafa	Tétouan, Beni Said	35°27.860'N/005°17.210'W	330
Aïn El Malâab	Chefchaouen, Parc National Talassemrane	35°05.509'N/005°09.443'W	1278
Daya Aïn Jdioui	Tanger-Assilah, Aïn Jdioui	35°34.074'N/005°55.499'W	5
Douar Kitane	Tétouan, Kitane	35°32.412'N/05°20.393'W	52
Douar Kouf	Allyene, M'diq-Fnideq	35°47.263'N/005°21.695'W	8
Golf Cabo Negro	Tétouan	35°64.8667'N/05°28.4266'W	2
Ksar Rimal	M'diq-Fnideq, Kabila	35°43.806' N/005°20.509'W	11
Larbaa Beni Hassan	Tétouan	35°19.1270'N/05°21.1840'W	608
Oued Aârate	Chefchaouen, Dardara	35°07.381'N/05°17.456'W	269
Oued El Kannar	Chefchaouen, Stehate	35°30.920'N/04°97.828'W	37
Oued Siffaou	Chefchaouen, Tougharine	35°11.609'N/005°18.402'W	278
Oued Tkaraâ	Larache, Jbel Bouhachem	35°16.063'N/05°25.829'W	959
Marabout Sidi Bouhadjel	M'diq-Fnideq, Restinga	35°43.596'N/005°24.517'W	77
Aïn Haouzi	Chefchaouen, Tougharine	35°11.213' N/005°17.144'W	480
Middle Atlas Mountains			
Aïn Arougo	Khénifra	32°55.387'N/05°34.081'W	118
Cascade Aïn Vittel	Ifrane, Aïn Vittel	33°33.682'N/005°07463'W	1555
Mchacha Aïn Vittel	Ifrane, Aïn Vittel	33°33.206'N/05°06.722'W	1585
Rabat-Salé-Kénitra Region			
Douar Ouled Ammar	Khmiss Rmila, Sidi Kacem	34°24.969'N/06°06.083' W	14



Figure 1. Rearing. a) in the wild from larvae found in litter; b) in the laboratory from organic matter from a barn in Douar Kitane.

RESULTS

Faunistic Records

Subfamily Beridinae

Genus *Chorisops* Rondani, 1856

Chorisops tibialis (Meigen, 1820)

Material examined: Rif: Tétouan, Douar Kitane, 1♂, 9.10.2016-18.10.2016, 8♂, 6♀, 18.10.2016 -31.10.2016, 5♂, 4♀, 31.10.2016 -14.11.2016, Malaise trap. First record from North Africa.

World distribution: Euro-Mediterranean species, present in Austria, Belgium, Cyprus, Czech Republic, England, France, Hungary, Ireland, Israel, Italy, Germany, Greece, Netherlands, Poland, Romania, Slovakia, Spain, Switzerland, Wales and Yugoslavia (Woodley, 2001) and North Africa (present work).

This is the first record of *C. tibialis* from North Africa. It was collected in the Rif in Tétouan at an altitude of 52 m a.s.l.

Biology: Stubbs and Drake (2014) summarize the British data on the larvae of *C. tibialis* and associate them with rotting plant material (damp wood-mould in rot holes in trees and grass tussocks) and according to Handlirsch (1883), the larva was found in June among leaves. The flight period in Europe runs from the second half of May till late September and adults are often found in or near woodland (Stubbs & Drake, 2014).

Chorisops tibialis, a species of fly known for its summertime flight period in Europe, has been studied extensively for its ecological behavior. Lebard et al (2020) documented its presence in Europe until late September, suggesting the possibility of two annual generations.

In Morocco, our research conducted indicates a notable deviation from this pattern, with the species displaying activity later in the year, particularly from October to mid-November.

Subfamily Pachygastrinae

Genus *Pachygaster* Meigen, 1803

***Pachygaster atra* Panzer, 1798**

= *Nemotelus ater* Panzer, 1798

Material examined: Rif: Tétouan, Beni Said, Aazafa 1♀, 20.5.2018, sweep net. New localities for Morocco.

World distribution: Species of Turanic-European-Mediterranean distribution covering Europe (although its distribution seems to be limited in part also in the southern regions), the Middle East, Anatolia, the Balkan and the Caucasus (Woodley, 2001) and North Africa (Yimlahi et al, 2017) and Morocco (Aazafa) (New site).

Moroccan distribution: *P. atra* was collected in two localities within the Rif region, spanning two different provinces: Tétouan (Yimlahi et al, 2017) and Tangier-Assilah, where it was discovered at altitudes of 330 m and 778 m above sea level, respectively

Biology: Adults are often present locally on shrubs in the sun, at the edge of forests. Swarming has been recorded several times (Szilády, 1932; Piet, 1950; Verrall, 1909) and when mentioned the swarms consisted of males. The latter also noted a mass occurrence on *Pteridium aquilinum* in England. The main flight period ranges from May to August, but adults can be found as early as April and as late as September. Larvae have been found in moist soil among the fallen leaves of *Juglans regia*, the decaying leaves of *Verbascum*, *Salvia aethiopsis* and others (Heeger, 1853; Dušek & Rozkošný, 1975).

In Morocco, the dates of collection of *P. atra* in Morocco fall within the flight period known from the literature, between April and May. The new locality is in a marshy

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humid environment of a complex of stream and saline pond, on soil characterized by irrigated agriculture where plum tree is the main cultivated fruit tree.

Subfamily Hermetiinae

Genus *Hermetia* Latreille, 1804

***Hermetia illucens* (Linnaeus, 1758) (Figs 2-4)**

Material examined: Rif: Tétouan, Douar Kitane, 13♂, 26 larvae, 1.7.2014, 20♀, 19 larvae, 17.7.2014, 1♂, 9♀, 104 larvae, 16.10.2015, 12 larvae, 24.5.2016, 19 larvae, 10.12.2016, 113 larvae. The adult collected by sweep net, 8♂, 14♀, 1.11.2015-17.12.2015, reared; Ksar Rimal, 1♀, 25.11.2018; Larbaa Beni Hassan, 1♂, 6.12.2020, sweep net; Chefchaouen, Dardara, Oued Aârate, 1♀, 10.7.2018, sweep net. Formally recorded from Morocco for the first time.

World distribution: Of almost cosmopolitan distribution, about between 45°N and 40°S, *H. illucens* is widespread in all zoogeographical regions. Apparently of American origin, it was spread worldwide through human activities. It should be noted that it was not recorded from North Africa until very recently when Koutsoukos and Kazilas (2021) added it to the Algerian list.

Only recently *H. illucens* was reported for Morocco based on a personal observation mentioned in Demetriou et al (2022), but no details were given. We here formally record *H. illucens* from Morocco for the first time and we thus add a new subfamily, the Hermetiinae, to the Moroccan stratiomyid fauna. It was collected in three localities in the provinces of Chefchaouen and Tétouan, in an altitudinal interval ranging from 11 m to 608 m.

Biology: Within its range *Hermetia illucens* is locally common on tree trunks and on garden plants in residential areas, and often seen resting on the walls and windows of houses (Callan, 1974). Larvae have been found in a wide range of decaying plant and animal materials; they are terrestrial scavengers (McFadden, 1967). European specimens have been collected from April 25 to November 8. Similarly, the Moroccan habitats where adults of *H. illucens* were found were particularly rich in decaying plant and manures. Males and females (Fig. 2a-b) were initially captured by sweep-netting. Larvae were found in manure (Figs. 3-4).

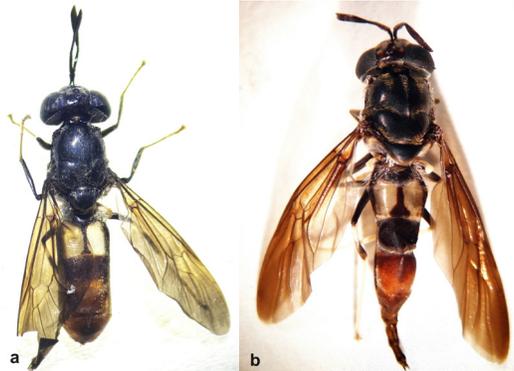


Figure 2. *Hermetia illucens* dorsal view; a) male, b) female.



Figure 3. Larvae of the black soldier fly, *Hermetia illucens*; in manure in Douar Kitane environment.



Figure 4. Larvae of the black soldier fly, *Hermetia illucens*; a) dorsal view, b) ventral view

Subfamily Sarginae

Genus *Chloromyia* Duncan, 1837

Chloromyia formosa (Scopoli, 1763)

= *Musca formosa* Scopoli, 1763

Material examined: Rif: Tétouan, Douar Kitane, 6♂5♀, 5.3.2017-24.4.2017, reared; Chefchaouen, Tougharine: Oued Siflaou (Fig. 5a), 1♀, 9.5.2017, sweep net; Tétouan, Beni Said: Aazafa 4♂, 1♀ (Fig. 5b), 20.5.2018, sweep net. New localities for Morocco.

World distribution: Species of Holarctic distribution encompassing Europe, Asia and North America. It stretches from central Scandinavia to northern Africa and eastwards to eastern Siberia. Almost all local lists of European Stratiomyidae include it. In North Africa it is known from all three Maghreb countries: Algeria, Tunisia and Morocco.

Moroccan distribution: In recent years it was found in 5 localities, distributed in the provinces of Tétouan and Chefchaouen in a considerable large altitudinal interval ranging from 52 m to 828 m a.s.l.

Biology: Larvae have been found in garden soil, compost, heaps of rotting grass, under stones and often in manure (Laurence, 1953; Brindle, 1965; Stubbs & Drake, 2014) and hibernation takes place in the larval stage. Adults are present in low grasslands, on the leaves of shrubs and in similar situations, usually in sunny places (Chandler, 1975). In the Alps the species is known up to 2270 m a.s.l. (Bezzi, 1918; Keiser, 1947). The flight period is mainly from April to August.

In Morocco, *Chloromyia formosa* is found in a wide range of habitats. Males and females were trapped in our Malaise trap, already in March so it was collected earlier than cited in the literature. In our study area, *Chloromyia formosa* has been found both in forest habitats and open spaces where it colonizes both humid environments of lotic character, rivers and streams, as well as lentic environments and marshy lands.

As mentioned in the literature (Rozkošný, 1982), adults were also collected feeding on flowers, for example *Euphorbia helioscopia* (Fig. 6).

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Figures 5. Moroccan habitat of *Chloromyia formosa*; a) Oued Siflaou, b) Oued Aazafa.



Figure 6. Moroccan habitat of *Chloromyia formosa* (Scopoli, 1763); Douar Kitane.

Subfamily Stratiomyinae

Tribe Oxycerini Enderlein, 1914

Genus *Oxycera* Meigen, 1803

Oxycera analis Wiedemann in Meigen, 1822

Material examined: Middle Atlas: Ifrane, Cascade Aïn Vittel (Fig. 7a), 2♂, 17.2.2016-21.4.2016, reared; Mchacha Aïn Vittel (Fig. 7b), 1♂, 17.2.2016-22.4.2016, reared. First record for North Africa.

World distribution: A relatively rare Palearctic species, distributed in Europe, Turkey and eastern Transcaucasia (Woodley, 2001) and North Africa (present work).

Moroccan distribution: The authors collected *O. analis* in the Middle Atlas, in the province of Ifran at 1585 m a.s.l., the first record for North Africa. This discovery greatly expands the range of the species and this is now the southernmost record for the species.

Biology: Adults generally are caught around ponds, as well as in marshes along streams and rivers. In the French Alps larvae were found at altitudes of 300 m to 1950 m a.s.l. in hygropetric situations (Vaillant, 1951). The reported flight period was June 1 to July 17.

In Morocco, during our surveys we found larvae on mosses and other plants that line the limestone-rich surroundings of the Aïn Vittel waterfall and the stream Mchacha Aïn Vittel nearby, a region of a very humid climate. In the laboratory we succeeded in rearing three males from larvae that were buried in Mossy substrate collected at these sites.



Figure 7. Habitat of *Oxycera pardalina* (Meigen, 1822) and *Oxycera analis* Wiedemann in Meigen, 1822 in Morocco: a) Cascade Ain Vittel; b) Mchacha Ain Vittel.

***Oxycera flava* (Lindner, 1938)**

= *Heraclina flava* Lindner, 1938

Material examined: Rif: Chefchaouen, Stehate, Douar El Kannar (Fig. 8), 1♂, 1♀ (Figs. 9-11), 24.4.2015, sweep net. New record for North Africa, with the male described for the first time.

Comment: New record for North Africa. So far, only the female of *O. flava* was described (from Andalusia, Spain) and there were no published records of the male of this species. We now describe it here, based on material collected in the Rif Mountains.

Female (Fig. 10): Head completely yellow, but only a dot slightly ocellar triangle is black, and below the middle of the forehead is black (see Rozkosny, 1982). The antenna is the same in both sexes. Abdominal pattern with broadly yellow lateral edges extends from the lateral edge of tergum 1 to the last tergum. The female mesonotum with narrow transversal mesonotal stripes that are connected to one another in front of the transverse suture and fused anteriorly; the yellow pattern is absent in male. Notopleura with widely yellow bands in both sexes. Legs and wings are the same in both sexes.

Male (Fig. 11a-c): Body length: 6 mm, wing 5.0 mm.

Head (Fig. 9a) slightly broader than thorax in dorsal view. Vertex black. Ocellar tubercle and ocelli brown, eyes contiguous, brown. Occiput black, only vertex with yellow spot behind ocellar tubercle. Eyes with sparse, short brown hairs. Frons black. Face yellow, with broad silvery white bands along the eye margins. Postocular area slightly swollen in lower half, densely silvery-white pubescence, the pubescence extending to the lower corner of the eye and joining with broad silvery white bands along the eye margins on face. Antenna about half as long as head; scape and pedicel yellow, flagellum light brown, the last flagellomere is slender, dark and slightly longer than the remainder of the antennae. Proboscis yellow.

Thorax (Fig. 11b, c) black. Scutellum and scutellar spines yellow, length of scutellar spines about two-thirds the length of the scutellum. Postpronotal lobes yellow. Notopleura is broadly yellow from postpronotal lobe to the postalar callus. Subnotopleural stripes very broad and extending to the anepimeron, the upper margin of katapisternum and the upper margin of the meron. Legs yellow, but the fore tibiae with a dark stripe on the dorsal surface and fore tarsi completely black; hind tibiae yellow with broad dark brown median ring; last three tarsal segments of mid and hind legs brown. Wing hyaline, R4 absent. Halteres light yellow.

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Abdomen (Fig. 11b, c) black with a broad undulating yellow margin Similar to that of female but not extending on to segments 1-2. Venter mainly yellow but sternites with brownish lateral patches at anterior margins.

Terminalia (Fig. 9a-d, 11b, c,) exposed, proctiger subtriangular, cerci elongate-oval, about 2-2.5 times as long as broad at middle; the epandrium is nearly equivalent in size to the proctiger at its midpoint. Genital capsule subquadrate, Median process of the synsternum separated with V-shaped indentation in the middle, which is not very deep. Gonostylus, the lower part of which is dilated, curves inward from the middle and gradually becomes thinner, ending in a pointed tip. The phallic complex is moderately short and broad, with parameres are separated and almost equal in length.

In males, distinguishing features between *Oxycera stigmosa* and *Oxycera flava* include the coloration of the flagellum and face. In *Oxycera stigmosa*, the flagellum of the antenna is black, whereas in *Oxycera flava*, it tends to be a lighter brown shade. Additionally, the face of *Oxycera stigmosa* is black, contrasting with the yellow face of *Oxycera flava*. Another distinguishing characteristic is observed in the spines of the scutellum: in *Oxycera stigmosa*, the spines are longer compared to the length of the scutellum, whereas in *Oxycera flava*, these spines are slightly shorter relative to the scutellum.

World distribution: This is only the third record of the species that until now was only known from Spain and France (Woodley, 2001) and recently from Corsica (Lebard & Hauser 2023). Consequently, this is also the first record for Morocco and thus North Africa. In the Rif it was collected in only one province (Chefchaouen), at an altitude of 37 m a.s.l.

Biology. The female *O. flava*, previously unknown, was collected along the El Kannar River (Fig. 8) in Morocco. This discovery offers the initial insights into the species' biology. The insect was found in the wet margins of the river's middle course, characterized by sand, pebbles, and moss-covered boulders. Adult activity appears limited to late April.



Figure 8. Moroccan habitat of *Oxycera flava* (Lindner, 1938): Oued El Kannar.

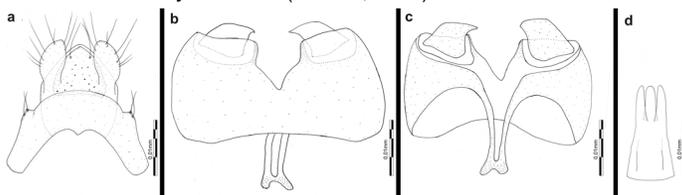


Figure 9. *Oxycera flava*, male genitalia. a) Epandrium, dorsal view; b) Synsternum, dorsal view; c) Synsternum, ventral view; d) Phallic complex. Scala bar: 0.01 mm.

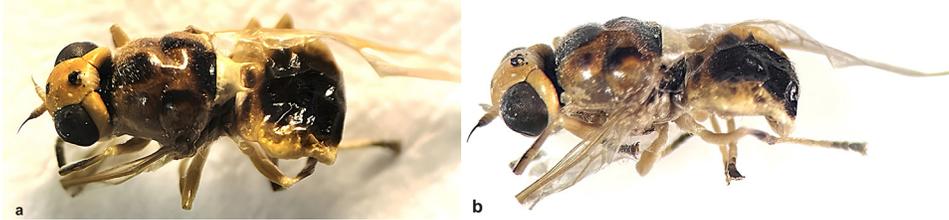


Figure 10. *Oxycera flava*, female: a) dorsal view; b) lateral view.



Figure 11. *Oxycera flava*, male a) ventral view of head; b) dorsal view; c) lateral view.

***Oxycera marginata* Loew, 1859**

Material examined: Middle Atlas: Ifrane, Cascade Aïn Vittel (Fig. 7a), 2♀, 17.2.2016-20.4.2016, reared; Mchacha Aïn Vittel (Fig. 7b), 1♀, 17.2.2016-21.4.2016, reared. First record for North Africa.

World distribution: This species was only known from Europe, having a Western Mediterranean distribution (Iberian Peninsula and southern Italy, including Sicily) and recently from Corsica (Lebard & Hauser 2023). The species is recorded for the first time for the fauna of North Africa.

Moroccan distribution: *Oxycera marginata* was now collected at two localities in Ifrane (Middle Atlas).

Biology: So far the biology was unknown except that it was a species active in early summer with collecting dates ranging from the beginning of June to 27 July (Rozkošný, 1983).

In Morocco, we have collected substrate particularly rich in mosses and other plants from the torrential habitat of the waterfall of Aïn Vittel and that of the streams that flow from it (Mchacha Aïn Vittel). This substrate contained several larvae and we successfully reared this species in the laboratory, according to the protocol described in Materials and Methods section of the 2021 thesis of the first author (Yimlahi, 2021).

These aquatic ecosystems of high altitudes (higher than 1500 m a.s.l.), seem to be the preferred habitat of *O. marginata* but also of *O. analis*, since the preimaginal stages of the two species coexist perfectly.

***Oxycera morrisii* (Curtis, 1833)**

Material examined: Middle Atlas: Ifrane, Cascade Aïn Vittel, 2♂, 17.2.2016-20.4.2016, reared; Mchacha Aïn Vittel, 1♂, 1♀, 17.2.2016-21.4.2016, reared; Aïn Arougo (Fig. 12), 1♀, 14.7.2016, sweep net. Recorded from Morocco for the first time.

World distribution: A western Palaearctic species, known from Europe, Algeria, Turkey and Israel.

Moroccan distribution: It is here recorded from Morocco for the first time. It was collected in two provinces in the Middle Atlas, Khénifra and Ifrane, at altitudes of 118 m and 1500-1585 m a.s.l., respectively.

Biology: Haliday (1857) found the larva among algae covering a dam of a watermill outlet that was continuously moistened by running water, and adults were found near places of larval development. Bertrand (1949) found the larvae among the wet moss in hygropetric situations, along with some other hygropetric larvae of Ceratopogonidae and Psychodinae. His collected material suggested a flight period between June 6 and July 25 but in the United Kingdom it was also found in August (Stubbs & Drake, 2014).

In Morocco, during our research an adult female was collected by sweeping herbaceous vegetation and rushes near a stream of Aïn Arougo, that also features the species larval habitat. The remainder of the material was obtained by rearing in the laboratory, at room temperature, from larvae living in the substrate of the Aïn Vittel waterfall (Fig. 7a) and that of Mchacha Aïn Vittel (Fig. 7b). It took a little over two months for the adults to hatch, from 17 February, the date of collection, until 20 April. As in the literature, the preimaginal stages of *O. morrisii* prefer slowly running water that is rich in algae and mosses.



Figure 12. Habitat of *Oxycera morrisii* in Morocco: Aïn Arougo.

***Oxycera pardalina* (Meigen, 1822)**

Material examined: Rif: Chefchaouen, Stehate, Oued El Kannar, 1♀, 15.8.2016, sweep net. New localities for Morocco.

World distribution: Species with European-Mediterranean distribution that extends east to the Caucasus and west to Morocco (Oued El Kannar) (New site).

Moroccan distribution: Our earlier samples from the Rif, from the provinces of Tétouan and Chefchaouen, and from the Middle Atlas (Ifrane), were the very first

citations of the species from North Africa (Yimlahi et al, 2017). In Morocco, *Oxycera pardalina* exploits altitudes ranging between 37 m and 1585 m a.s.l.

Biology: According to Rozkošný (1983) the larvae prefer calcareous waters and usually are met in hygropetric conditions on wet stones and rocks and in wet moss in and near springs and streams. Vaillant (1951) found larvae in various sites from 200 to 2500 m a.s.l. in the Alps. Adults were collected on *Heracleum sphondylium* and *Saxifraga aizoides* (Keiser, 1947). The flight period runs from May to August (Rozkošný, 1983; Stubbs & Drake, 2014).

In our study area, that of previous (Yimlahi et al, 2017) and current studies, this species is found in a wide range of habitats, but always in environments with aquatic ecosystems of running water, from very fast running (waterfalls and upper wadis) to slow (lower rivers and streams). The substrates hosting the preimaginal stages of *O. pardalina* were amassed in beds, mostly dominated by rocks and pebbles, and rich in mosses and algae.

During our sampling we located this species in two geographical regions, the Rif (5 sites in 2 provinces) and the Middle Atlas (2 sites in the province of Ifrane), in a wide altitudinal interval, ranging from 50 m to 1674 m a.s.l. (Yimlahi et al, 2017). In the northern Moroccan region, the species also seems to frequent high-altitude forest habitats, where it was collected near a spring stream that crosses the Talassemrane fir forest characterized by the presence of boulders lined with mosses, pebbles, sand and silt in places.

When we reared material in the laboratory, the emergence of adults took 40-60 days. Adult emergence from material collected on February 17 in the Middle Atlas, was earlier and continued to April 20. In the Rif, on the other hand, for rearing experiments that began later, in April and May, the emergences were from the first fortnight of June onwards. The flight period in Morocco runs from the end of April to August, a wider range than reported in literature (see above), both starting earlier and ending later.

***Oxycera trilineata* (Linnaeus, 1767)**

= *Hermione bucheti* Séguy, 1930

Material examined: Rif: Tanger-Assilah, Aïn Jdioui Daya, Aïn Jdioui, 1♂, 28.3.2019-18.5.2019, reared, 1♂, 28.3.2019-20.5.2019, reared. New localities for Morocco.

World distribution: The commonest species of the genus and known to be present throughout the Palaearctic region, from Western Europe (Mason et al, 2009), and Northern Africa to Central Asia (Hauser, 2014) and China. In Northern Africa, the species is known from Algeria, Morocco.

Morocco distribution: Tangier (Séguy, 1930), and new site Daya Aïn Jdioui (Yimlahi et al, 2017).

The new records, we collected larvae in the Rif at one site in Tanger-Assilah, at an altitude of 5 m a.s.l.

Biology: Larvae are found in a wide range of wet habitats, including banks of streams, pools, ponds, marshes, fens, seepages and springs (Rozkošný, 1983; Stubbs & Drake, 2014). In Algeria larvae were found in mud and among calcium carbonate

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deposits on wet rocks up to 1200 m a.s.l. (Vaillant, 1952). Adults are usually mostly found near the larval habitat from early June to early September (Stubbs & Drake, 2014).

We ourselves spotted it on a small saline daya (depression of ground filled by overflow water from river), eutrophicated, invaded by duckweed and fed by a stream near the Atlantic coast, but at a very low altitude. We successfully reared the species in the laboratory. The collected substrate, brought back for this purpose, was loamy, rich in macrophytes and had been taken from the edges of a bog and a daya.

Tribe Stratiomyini Latreille, 1802

Genus *Odontomyia* Meigen, 1803

***Odontomyia limbata* (Wiedemann, 1822)**

= *Stratiomys limbata* Wiedemann in Meigen, 1822

Material examined: Rif: Chefchaouen, Parc National Talassemtane, Aïn El Malâab, 1♂, 2♀, 17.5.2014, 1♂, 4.7.2020. New localities for Morocco.

World distribution: Species of Western Mediterranean distribution. In North Africa, the species is known from three western Maghreb countries along the Mediterranean.

Moroccan distribution: it had been recorded from Tangier and the Middle Atlas (Becker & Stein, 1913; Séguy, 1930; Dušek & Rozkošný, 1962).

Presently, we can add seven sites for *Odontomyia limbata* (Yimlahi et al, 2017), considerably expanding its range in two provinces in the Rif (Chefchaouen and Larache), in a wide altitudinal range from 600 m to 1278 m a.s.l.

Biology: Until now, the specifics of the biology of *Odontomyia limbata* are unknown.

In Morocco, *O. limbata* is associated with aquatic environments of the lentic type. It was collected near peat bogs, ponds. We also collected it near lotic habitats (streams).

In the majority of these sites silt with sparse gravel, cobbles and some boulders was the dominant substrate. These sites were located between pine forests, oak forests and meadows.

The species was captured near a spring stream running over a limestone substrate through *Abies marocana* fir forest, the banks and coarse substrate of which were lined with moss in places.

Genus *Stratiomys* Geoffroy, 1762

***Stratiomys longicornis* (Scopoli, 1763)**

= *Hirtea longicornis* Scopoli, 1763

Material examined: Rif: Chefchaouen, Parc National Talassemtane: Aïn El Malâab 1♂, 17.5.2014, sweep net; Rabat-Salé-Kénitra Region: Douar Ouled Ammar (Fig. 13), 1♂, 10.6.2017, sweep net; Tétouan, Golf Cabo Negro, 1♀, 15.5.2020, hand hunting. New localities for Morocco.

World distribution: *S. longicornis* is widely distributed in the Palaearctic region, including Europe, Transcaucasia, North Africa, Central Asia, Mongolia and Russia. In North Africa, the species is known from Algeria, Tunisia, Egypt, Morocco (Badrawy, 2006).

Moroccan distribution: Séguy (1930) recorded the species from Casablanca. New records it was now netted in three localities (Chefchaouen: Aïn El Malâab, Cabo Negro and Sidi Kacem: Douar Ouled Ammar) at 1278 and 14 m a.s.l., respectively.

Biology: The larvae develop among aquatic vegetation in stagnant water in saline habitats, like ponds near the coast, salt marshes and saline inland habitats, while they can hibernate in wet earth (Rozkošný, 1982). Adults can be found on vegetation and flower-visiting near larval habitat. They were recorded from flowers of *Crataegus*, *Chaerophyllum*, *Artemisia*, *Evonymus*, *Ferulago* and *Petasites* (Schiner, 1855; Speiser, 1910; Szilády, 1932; Lindner, 1938; Weinberg, 1972). Foremost it appears to be a lowland species preferring highly saline habitats, active from mid-May to late August.

In Morocco, our findings on the biology of *S. longicornis* mostly corroborate data in the literature, although we did not capture them after June. The males were swept from herbaceous plants near stagnant water at low altitudes, for example at the Oued Sebou, Douar Ouled Ammar (Fig. 13). The female was hand-caught, early in the morning, whereas it was found resting on a motorcycle near a small lake in Cabo Negro.



Figure 13. Habitat of *Stratiomys longicornis* (Scopoli, 1763) in Morocco: Douar Ouled Ammar.

Subfamily Nematelinae

Genus *Nemotelus* Geoffroy, 1762

Nemotelus cingulatus Dufour, 1852

= *Nemotelus consimilis* Becker, 1915

Material examined: Rif: Larache, Jbel Bouhachem, Oued Tkaraâ, 1♂, 1♀, 22.5.2017, sweep net; Aïn Haouzi, 1♀, 9.5.2017, sweep net. New localities for Morocco.

World distribution: A species of the Western Mediterranean, *N. cingulatus* is known to be present in Spain, southern France and North Africa.

In North Africa, it known from Tunisia (type locality Korbons [=Qurbus]; Becker, 1915), Algeria (Lindner, 1931), Morocco.

Moroccan distribution: (Rozkošný, 1983; Yimlahi et al, 2017), and new localities (Oued Tkaraâ and Aïn Haouzi) in the present work.

If we combine our previous (Yimlahi et al, 2017), and the current studies research we collected *N. cingulatus* again, at four sites ranging from 124 m to 959 m a.s.l.

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Biology: Rozkošný (1983) recorded the flight period as April 26 to May 17 with adults being captured up to an altitude of 1000 m a.s.l. in Spain.

In Morocco, during our research we found the species flying a bit later (22 May) and in both lentic and lotic aquatic habitats. It was collected near peat bogs and ponds of the Daya Afrate. We also collected it in the upper reaches of Oued Tkaraâ in Jbel Bouhachem, running through an oak forest, and around Oued El Kouf. These last two sites are characterized by the presence of boulders lined with moss, sand, pebbles and silt in places.

***Nemotelus longirostris* (Wiedemann, 1824)**

Material examined: Rif: Chefchaouen, Tougharine, Oued Siflaou, 1♀, 9.5.2017, sweep net; Douar Kouf, 1♂, 1♀ (Fig. 14), 19.5.2019, sweep net. New localities for Morocco.

World distribution: Western Mediterranean species, known in Europe only from France and Spain, and in North Africa, from the three Maghreb countries: Algeria (Lindner 1931), Tunisia (Becker 1906) and Morocco.

Moroccan distribution: In our country, *N. longirostris* was mentioned in the Rif in Tangier (Wiedemann, 1824; Becker & Stein, 1913; Séguy, 1930), Fès (Lindner, 1931), and new localities (Oued Siflaou and Douar Kouf) in the present work.

We can now confirm the presence of *N. longirostris* in the Rif, but also in the province of Chefchaouen (a female in Tougharine) and in the province of M'diq-Fnideq (a male and a second female in Douar Kouf), at altitudes of 8 m and 480 m a.s.l., respectively.

Biology: There is no information on the biology of this species. It was only collected in May (Rozkošný, 1983; present data).

In Morocco, the adults were collected in environments, rich in vegetation with plants like *Anacyclus clavatus*, *Anacyclea radiatus*, *Pulicaria odora*, *Andryala integrifolia*, *Ficus carica*, *Olea europaea*, *Lavandula stochae* and *Dittrichia viscose*.



Figure 14. Moroccan habitat of *Nemotelus longirostris* (Wiedemann, 1824): Douar Kouf.

***Nemotelus nigrifrons* Loew, 1846**

= *Nemotelus tomentosus* Becker, 1906

Material examined: Rif: M'diq-Fnideq, Restinga, Marabout Sidi Bouhadjel 19.5.2019 (Fig. 15), 17♂6♀, sweep net. New locality for Morocco.

World Distribution: Widely spread in the Mediterranean, known from Europe (Italy) and North Africa: Algeria (Becker, 1906), Tunisia (Lindner, 1936-1938), Libya (Rozkošný, 1983).

Moroccan distribution: Morocco (Becker, 1906; Yimlahi et al, 2017), and new locality (Marabout Sidi Bouhadjel) in the present work.

Biology: There is no information on the biology of this species. It was collected between the end of April and 3 August (Rozkošný, 1983).

In Morocco, the species was encountered near aquatic environments of a lentic nature; most specimens were caught on vegetation growing around marshes with mud rich in organic matter and with dominance of *Typha* and rushes at the Marabout Sidi Bouhadjel (Fig. 15). However, specimens of this species have also been found in the vicinity of running waters (in lotic ecosystems).



Figure 15. Moroccan habitat of *Nemotelus nigrifrons* Loew, 1846: Marabout Sidi Bouhadjel.

DISCUSSION

Our study presents an extensive examination of Stratiomyidae specimens collected from various regions of Morocco between 2014 and 2020. The findings significantly contribute to the understanding of the distribution, biodiversity and Biology of these insects in Morocco.

The new findings given in this study enrich the number of Stratiomyidae with six species, raising this number to 46 species of soldier flies known from Morocco.

In a previous study (Yimlahi et al, 2017) a checklist of soldier flies species recorded from the North African countries (Morocco, Algeria, Tunisia, Libya and Egypt) was presented, based on both literature records and material newly collected at that time in Morocco. Four subfamilies (Stratiomyinae, Sarginae, Nemotelinae, and Pachygasterinae), and twelve species from five genera were collected and recognized in Morocco. Six species: *Pachygaster atra* (Panzer, 1798), *Oxycera pardalina* (Meigen,

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1822), *Nemotelus danielssoni* (Mason, 1989), *Oxycera terminata* (Meigen, 1822), *Nemotelus atriceps* (Loew, 1856) and *N. maculiventris* (Bigot, 1861) were reported for the first time in Morocco of which no less than four species (*P. atra*, *O. pardalina*, *O. terminata* and *N. danielssoni*) were new records for North Africa. The number of soldier flies known from Morocco was 40.

New data about Moroccan soldier flies (Stratiomyidae) are presented in the present study that discusses 15 species. Six species are recorded as new to the fauna of Morocco, four of which, *Oxycera analis* Wiedemann in Meigen, 1822, *O. flava* (Lindner, 1938), *O. marginata* Loew, 18959, and *Chorisops tibialis* (Meigen, 1820) are new records for North Africa. *Oxycera morrisii* (Curtis, 1833) is recorded for the first time in Morocco. *Hermetia illucens* (Linnaeus, 1758) was recently reported based on a personal communication without details but is now also formally added. It therefore now adds a new subfamily to the Moroccan fauna, the Hermetiinae. Notes on taxonomy, distribution and biology of the species are added. The male of *O. flava* is described and illustrated.

It is also important to note that in the previous study mentioned (Yimlahi et al, 2017), several species (*Pachygaster atra*, *Chloromyia formosa*, *Oxycera pardalina*, *Nemotelus cingulatus* and *Odontomyia limbata*) were referenced but without having studied their biology. In the present work, we provide detailed information about the biology of these species. Moreover, to enrich the content of this article, we have referenced both the previous collection sites and the current ones, providing a broader context for our discussion.

The inclusion of these new records enhances our understanding of the biogeography of Stratiomyidae in the Mediterranean region. The presence of species with Euro-Mediterranean distributions, such as *Chorisops tibialis* and *Chloromyia formosa*, highlights the biogeographical connections between North Africa and Europe.

The observed seasonal activity patterns of Stratiomyidae species in Morocco reveal minor variations compared to their counterparts in Europe. For example, the flight periods of certain species, such as *Chloromyia formosa* and *Oxycera pardalina*, appear to be influenced by climatic conditions, with activity extending into later months than reported in European populations.

The observed differences in the seasonal activity of *Chorisops tibialis* between Europe and Morocco raise intriguing questions about the factors driving these variations. One plausible explanation is the dissimilarity in climatic conditions between the two regions. Morocco's warmer climate, especially in late autumn, could provide favorable conditions for extended activity periods compared to the cooler European environment. Additionally, ecological factors such as host availability and habitat suitability might also play a role in shaping the seasonal behavior of *Chorisops tibialis* in different regions. This divergence in seasonal activity prompts an investigation into the potential influence of climatic variations between European and Moroccan environments.

The comprehensive faunistic records presented in this study significantly contribute to our knowledge of Stratiomyidae diversity and distribution in Morocco. The findings provide valuable baseline data for future research on the ecology, biogeography, and conservation of these important insects in North Africa.

CONCLUSION

Accounts of distribution and biology of 15 species of the Stratiomyidae of the Moroccan fauna are presented. *Chorisops tibialis*, *Oxycera analis*, *O. flava* and *O. marginata* are not only recorded from Morocco for the first time, but also from North Africa. *Oxycera morrisii* and *Hermetia illucens* are reported for the first time in Morocco. As a result of this work, the present number of soldier flies known from Morocco has been raised to 46. In general, the data we could obtain on the biology of species in Morocco is very similar to the known biological information from Europe except for some longer flight periods that might be the result of different climatological conditions in Morocco.

ACKNOWLEDGMENTS

We would like to thank Dr Younes El Harym (LESCB URL-CNRST N°18, FS, Abdelmalek Essaadi University, Tétouan, Morocco), for sharing with us the photo of *Chloromyia formosa* in its Moroccan habitat.

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Alternating Low and High Temperatures Increases the Hatching Rate of *Eupolyphaga sinensis* (Blattaria: Polyphagidae) Eggs

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ABSTRACT

The hatching rates and developmental durations of *Eupolyphaga sinensis* Walker eggs were studied under variable combinations of low temperatures (5 °C and 10 °C) and high temperatures (25 °C and 30 °C). Our findings were intended to provide new evidence on the effect of temperature on the hatching of insect eggs. The results showed that both low temperatures and high temperatures significantly affected the hatching rate and time of *E. sinensis* eggs. Further analysis revealed a significant interactive effect between low temperatures and high temperatures. The 5 °C + 30 °C treatment achieved the highest egg hatching rate (77.33%) and the shortest hatching time (44.11 ± 1.55 d), which was significant compared with three other temperature treatments. The effects of high temperatures treatments on the hatching rate and the hatching time were more significant than that in the low temperature treatments. Hatching began earlier at 5 °C + 30 °C and 10 °C+30 °C temperatures, and single-day hatching first increased rapidly before declining afterwards within a short period. Comparatively, the hatching of eggs under the (5 °C+25 °C) and (10 °C+25 °C) temperatures was initially significantly delayed, including the peak single-day hatching rate. Under these treatments, the hatching rate tended to be gradual in terms of the temporal dynamics without showing any prominent peak. These results showed that low and high temperatures significantly affect the hatching rates of *E. sinensis* eggs. This study provided basis for the large-scale breeding of *E. sinensis*.

Keywords: Egg hatching, developmental duration, *Eupolyphaga sinensis*, variable temperature.

Li, Y., Guo, K., Zhang, C., Fu, W., & Chen, Z. (2024). Alternating low and high temperatures increases the hatching rate of *Eupolyphaga sinensis* (Blattaria: Polyphagidae) eggs. *Journal of the Entomological Research Society*, 26(?), 113-121.

Received: November 19, 2023

Accepted: March 20, 2024

INTRODUCTION

For most insects, eggs are the first developmental stage, and they directly affect the post-embryonic development of insects. In this sense, the development of insect eggs occupies a particularly critical place in research on insect ontogeny (Heming, 2003). Apart from adult foods, environmental factors, especially temperature, are the main factors affecting the development of insect eggs (Raza et al, 2015; Harrison, Woods, & Roberts, 2012; Gillespie, Picker, Dallas & Day, 2018). In particular, in middle and high-latitude regions with noticeable seasonal changes, the germination and hatching of insect eggs are highly affected by low temperatures and high temperatures (Liu et al, 2020). Exploring the effects of temperature change on the hatching of insect eggs not only provides a theoretical basis for the large-scale breeding of insects but is also a great reference for an in-depth understanding of the dynamic relationship between insect development and temperature.

Eupolyphaga sinensis Walker (Blattaria: Polyphagidae) is a traditional medicinal insect and has been included in the list of traditional Chinese medicine in the Pharmacopoeia of the People's Republic of China (Xie et al, 2020; Kim, Jim, Park, & Song, 2022). In recent years, studies have revealed that *E. sinensis* are rich in proteins, unsaturated fatty acids, fat-soluble vitamins, and a variety of nutrients, including amino acids and trace elements, essential for the human body (Zhang et al, 2014). Inspired by the folk eating experience, people have processed this insect into food (Li, Yan, & Guo, 2000). The dual use of *E. sinensis* as medicine and food has driven its market demands to continue increasing year by year. While the artificial culture technology of *E. sinensis* rearing is becoming increasingly advanced, the low hatching rate of its oothecae remains a notable challenge, particularly in large-scale culture (Li, Duan, Wu, Wang, & Wu, 2003; Jin, Tang, Wang, Wu, & Wu, 2005; Jin et al, 2006; Jin, Wu, Tang, Wang, & Wu, 2007; Wang & Liang, 2017). Regarding factors affecting the hatching of *E. sinensis* eggs, existing studies have only reported the hatching of *E. sinensis* eggs in natural environments or under different constant temperatures and soil moisture contents (Li, Tang, Zhang, Wu, & Wu, 2003; Jin et al, 2007; Wang & Liang, 2012, 2017). To date, the effect of temperature change on the hatching of *E. sinensis* eggs remains unclear. In the present study, we investigate the effects of different combinations of low-temperature (germination temperatures) and high-temperature (hatching temperatures) on the hatching of *E. sinensis* eggs. Our results deepen our understanding of the effect of varied temperatures on the hatching of *E. sinensis* eggs in natural environments, providing scientific guidance for the large-scale breeding of *E. sinensis*.

MATERIALS AND METHODS

Insect source

E. sinensis used in our experiment were provided by Yuchen Special Culture Base in Bozhou, Anhui Province. The same batch of newly emerged adults was reared

Alternating Low and High Temperatures Increases the Hatching rate of E. sinensis

at a room temperature of 25 ± 2 °C under a soil moisture content of about 20% (Wang & Zhang, 2000) until a large number of female adults had oothecae hanging on their abdomens. Before the experiment, 720 oothecae, characterized by regular appearance, bright shells, and even size, were selected from the oothecae laid on 21 July 2021. They were soaked in a 4% neutral formaldehyde for disinfection, rinsed with distilled water, and dried for use later (Jin et al, 2005).

Nest mud (hatching soil)

Nest mud (hatching soil) was prepared from humus soil, cow dung, and rice bran by mixing the three in a ratio of 1:1:1 (Jin et al, 2007).

Temperature treatments

The factorial experimental design (2×2) was adopted to set up four experimental treatments; two low-temperatures (5 °C and 10 °C) and two high-temperature (25 °C and 30 °C) treatments: (i) low-temperature at 5 °C + high-temperature at 25 °C (5 °C + 25 °C); (ii) low-temperature at 10 °C + high-temperature at 25 °C (10 °C + 25 °C); (iii) low-temperature at 5 °C + high-temperature at 30 °C (5 °C + 30 °C); (iv) low-temperature at 10 °C + high-temperature at 30 °C (10 °C + 30 °C).

The low temperature of 5 °C was achieved using a refrigerator (model: BCD-215STPD; manufacturer: Qingdao Haier Co., Ltd.). The low temperature of 10 °C and the high temperature of 25 °C and 30 °C were provided by a microcomputer artificial climate chamber (model: SPX-400; manufacturer: Shanghai Boxun Medical Biological Instrument Corp.). The temperature ranges were as follows: 5 °C \pm 0.5 °C, 10 °C \pm 1.0 °C, 25 °C \pm 1.0 °C, 30 °C \pm 1.0 °C. Each experimental treatment was composed of three replicates of 60 oothecae (one day old). The replicates were conducted in different refrigerators and chambers. The experimental oothecae were placed in a plastic square box (25.0 \times 25.0 \times 15.0 cm in size) containing hatching soil (about 12.0 cm thick) under alternating low and high-temperature treatments. The boxes were first placed in the proper set refrigerator and microcomputer artificial climate chamber for seven days at low-temperature, and then transferred to the properly set microcomputer artificial climate chamber for high-temperature treatment until the completion of egg hatching. The relative humidity of hatching soil was maintained at $25 \pm 5\%$ during the experiment by spraying water three times every day.

Hatching rate

The number of hatched eggs was regularly recorded daily at 14:00. It started from the appearance of the first nymph in the oothecae of each box and ended when no new nymph was observed (when no new nymph hatched from the oothecae for five consecutive days, egg hatching would be deemed as completed).

Data analysis

A homogeneity test was conducted each time before the statistical analysis without data conversion. Two-way ANOVA was used to test the effects of temperatures and high

temperatures on the hatching of *E. sinensis* eggs. One-way ANOVA was performed to compare the effects of different temperature treatments on the developmental duration, hatching rate, and single-day hatch rate of *E. sinensis* eggs. All data were analyzed using SPSS version 25.0. Continuous normally distributed data were expressed as mean \pm standard error ($X \pm SE$).

RESULTS

Effects of different temperatures on the cumulative hatching rate of *E. sinensis* eggs

The results indicated that the cumulative hatch rate of *E. sinensis* eggs varied significantly across four variable temperature combinations ($F = 580.718$, $P < 0.01$; Fig. 1). The highest hatching rate occurred under the 5 °C + 30 °C temperature (77.33 \pm 2.52%), followed by the 10 °C + 30 °C temperature (66.00 \pm 2.65%), the 5 °C + 25 °C treatment (23.33 \pm 1.53%), and the 10 °C + 25 °C treatment (15.00 \pm 2.00%) in this order. There was no difference in the number of nymphs hatched from each ootheca under different treatment conditions. The results indicated that there was a strong interaction between low and high temperatures, which significantly affected the hatching rate of *E. sinensis* eggs. Particularly, low temperatures had significant effect on the hatching rate of *E. sinensis* eggs ($F = 61.239$, $P < 0.01$). At the same combined high temperature (25 °C or 30 °C), the egg hatching rate at the combined low temperature of 5 °C was significantly higher than that at 10 °C ($P < 0.01$). At the same time, high temperatures also significantly affected the hatching rate of *E. sinensis* ($F = 1,785.332$, $P < 0.01$). At the same combined low temperature (5 °C or 10 °C), the egg hatching rate at the combined high temperature of 30 °C was significantly higher than that at 25 °C ($P < 0.01$). The results showed that the combined temperature (5 °C + 30 °C) significantly increased the hatching rate of *E. sinensis* eggs.

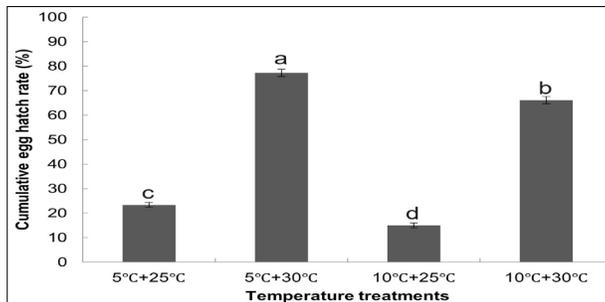


Figure 1. Effects of different temperatures on the cumulative hatching rate of *E. sinensis* eggs. Different lowercase letters above the bars indicate significant differences in cumulative hatching rate ($P \leq 0.05$, Tukey's test).

Developmental durations of *E. sinensis* eggs under different temperature treatments.

Oothecae treated at low temperatures for seven days (starting and ending time: 22:00, 21 July 2021–22:00 to 28 July 2021) were transferred to a microcomputer artificial

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climate chamber for high-temperature treatment on 28 July 2021 at 22:00. Under the 5 °C + 30 °C treatment, the first oothecae hatched on 31 August 2021, and no more ootheca hatching occurred after 8 September. The hatching period was 9 d, and the average developmental duration of eggs was 44.11 ± 1.55 d (Range: 41 – 49 d). Under the 10 °C + 30 °C treatment, oothecae hatching began on 2 September 2021, and no more hatching was observed after 9 September. The hatching period was 8 d, and the average developmental duration of eggs was 45.31 ± 1.57 d (Range: 43–50 d). Under the (5 °C + 25 °C) treatment, the oothecae began to hatch on 13 September 2021, and there was no hatching occurred after 27 September. The hatching period was 12 d, and the average developmental duration of eggs was 58.72 ± 3.78 d (Range: 54 – 65 d). Under the 10 °C + 25 °C treatment, oothecae began to hatch on 16 September 2021, and no more ootheca hatching was observed after 24th September. The hatching period was 9 d, and the average developmental duration of eggs was 60.97 ± 2.87 d (Range: 57 – 65 d). The results indicated that the developmental duration of *E. sinensis* eggs varied significantly across different temperature treatments ($F = 422.774$, $P < 0.05$; Fig. 2). Compared with low temperatures, high temperatures more significantly affected the developmental rate of eggs. The developmental duration of eggs at 30 °C was significantly shorter than 25 °C ($P < 0.01$).

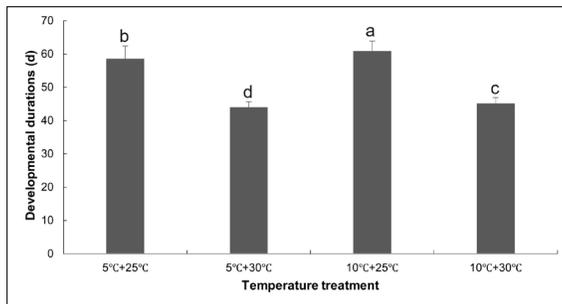


Figure 2. Developmental durations of *E. sinensis* eggs under different temperature treatments. Different lowercase letters above the bars indicate significant differences in the developmental durations ($P \leq 0.05$, Tukey's test).

Temporal dynamics of the egg hatch rate of *E. sinensis* under the same variable-temperature treatment.

The egg hatching rate fluctuated significantly from day to day under a given temperature (Fig. 3 and Fig. 4). With the extension of hatching time, the egg hatching rate of *E. sinensis* under the 5 °C + 30 °C and 10 °C + 30 °C first increased rapidly, then started to decline. Under 5 °C + 30 °C, the peak single-day hatching rate of eggs ($25.56 \pm 1.15\%$), counted from the beginning day of hatching ($F = 187.858$, $P < 0.001$; Fig. 4: A), occurred on the 4th day. Under 10 °C + 30 °C, the peak single-day hatching rate of eggs ($17.22 \pm 0.58\%$), counted from the beginning day of egg hatching ($F = 162.890$, $P < 0.001$; Fig. 4: C), occurred on the 5th day. In comparison, the eggs under 5 °C + 25 °C and 10 °C + 25 °C displayed a gradual hatching rate and had no pronounced peak hatching day. Under the 5 °C + 25 °C temperature, the peak

single-day hatching rate of eggs ($3.89 \pm 0.58\%$), counted from the beginning day of egg hatching ($F = 4.111, P = 0.002$; Fig. 4: B), occurred on the 14th day. Under the $10^\circ\text{C} + 25^\circ\text{C}$ temperature, the peak single-day hatching rate of eggs ($2.78 \pm 0.58\%$), counted from the beginning of egg hatching ($F = 3.551, P = 0.012$; Fig. 4: D), was observed on the 22nd day.

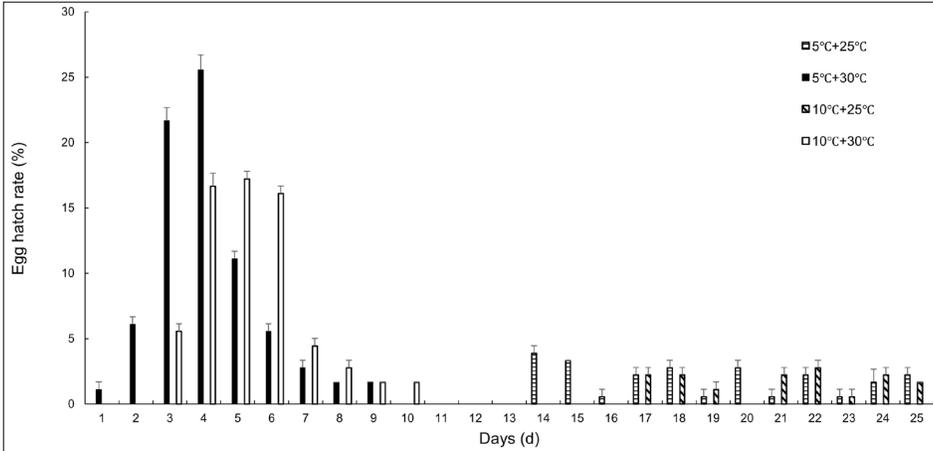


Figure 3. Single-day hatching rate of *E. sinensis* eggs under different temperature treatments. “Day 1” indicated the beginning day of egg hatching, the same below. ns: no significance. Asterisks above the bars indicate a significant difference in Single-day hatch rate ($P \leq 0.05$, Tukey’s test).

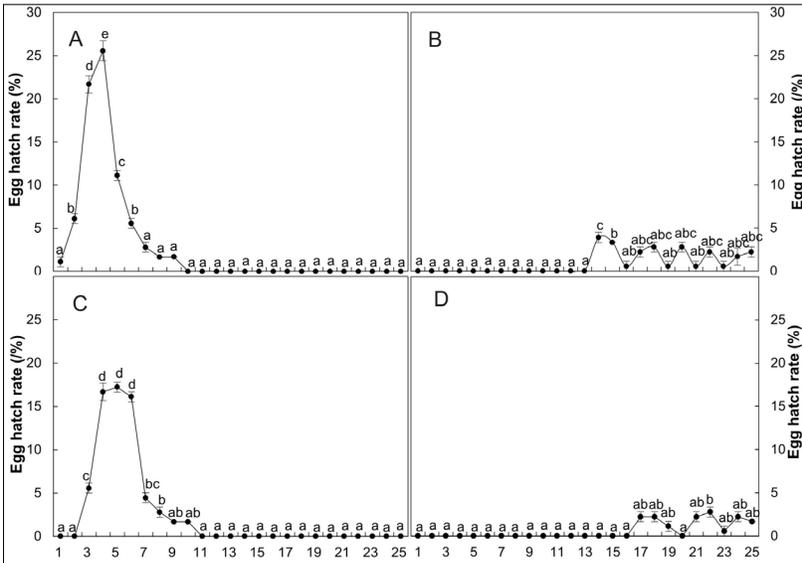


Figure 4. Temporal dynamics of the egg hatching rate of *E. sinensis* under different temperature treatments. A: $5^\circ\text{C} + 30^\circ\text{C}$; B: $5^\circ\text{C} + 25^\circ\text{C}$; C: $10^\circ\text{C} + 30^\circ\text{C}$; D: $10^\circ\text{C} + 25^\circ\text{C}$. Different lowercase letters indicate significant differences in the hatching rate ($P \leq 0.05$, Tukey’s test).

DISCUSSION

The low hatching rate of oothecae has remained a major constraint in the large-scale artificial culture of *E. sinensis*. According to the survey by Li et al. (2003), some farmers had complained of the extremely low hatching rate of oothecae (30 – 40%), which fell far short of the promise (80 – 90%) by some companies. Jin et al (2007) reported that the average hatching rate of *E. sinensis* oothecae under a temperature of 30 °C and a relative humidity of 95% was $64.7 \pm 6.7\%$. A related study by Wang & Liang (2017) showed that the hatching rate of *E. sinensis* oothecae peaks at 26 °C and 30 °C, reaching 56.5% and 52.8%, respectively. Our findings revealed that the peak hatching rate of *E. sinensis* oothecae occurs in 5 °C + 30 °C treatment, reaching $77.33 \pm 2.52\%$. In production practice, oothecae are first incubated at a low temperature of 5 °C for 7 d and then transferred to a high temperature of 30 °C to hatch. In theory, this approach can significantly improve the hatch rate of *E. sinensis* oothecae.

Temperature change affects the hatching rates of insect eggs and the survival rates of larvae, pupae, and adults (Pan, Chen, Xiao, Ji, & Xie, 2014). Compared with constant temperatures, variable temperatures can improve the survival rate of insects and prolong their survival time. For example, the hatching rate of *Gryllus bimaculatus* egg at a constant temperature of 20 °C is only 18% but rises to 49 – 65% under the diurnally alternating temperatures condition of 8 h (8.00 to 16.00) at 28 °C: 16 h at 16 °C (Behrens, Hoffmann, Kempa, & Merkel-wallner, 1983). The emergence rates of *Trichogramma japonicum*, *Trichogramma Conflum*, and *Trichogramma nubilale* improved after variable temperature treatment relative to their counterparts incubated under thermophilic conditions only (Zhu & Zhang, 1987). The emergence rate of *Aphidius gifuensis* after variable-temperature treatment is higher than that under a constant low temperature. Thus, the variable-temperature storage technology may be more suitable for the breeding and release of *A. gifuensis* (Tang et al, 2011). However, some studies have shown that low temperatures reduce the hatching rate of insect eggs. For example, Liu et al (2020) reported that low temperatures (4 °C and 7 °C) significantly lowered the egg hatch rate of *Acheta domesticus*. The authors believe that low temperature reduces the metabolism in *A. domesticus* eggs, making it impossible for them to either form a structure resistant to low temperature or to undergo complete embryonic development. Consequently, the egg-hatching rate declines under low temperatures. According to the findings of this study, low temperatures (5 °C and 10 °C) significantly increased the hatching rate of *E. sinensis* eggs. Clearly, the effect of temperature change on the hatching of insect eggs varies significantly across different species.

Generally, our findings concluded that low temperatures and high temperatures both significantly affect the hatching of *E. sinensis* eggs. Specifically, compared with low temperatures, high temperatures more significantly affected the hatching rate of *E. sinensis* eggs, which generally increased the hatching rate. Studying the effect of temperature change on the embryonic development and hatching of insect eggs provides solid evidence on the artificial breeding of insects. It should be pointed out

that, in addition to temperature, other abiotic factors, such as humidity, photoperiod, latitude, and altitude, may also affect the hatching of *E. sinensis* eggs to varying degrees. Moreover, a strong interaction and synergy may exist between them and temperature. Therefore, it is indispensable to identify the biotic and abiotic factors affecting the hatching of *E. sinensis* eggs, as well as the interaction and synergy between various environmental factors in the future.

ACKNOWLEDGEMENTS

This work was supported by the Science and Technology Program of Yunnan Province (202301AU070134).

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Description of a New Species of *Panotrogus* Reitter, 1902 (Coleoptera: Scarabaeidae: Melolonthinae) from Uttarakhand, India

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ABSTRACT

Panotrogus Reitter, 1902 is genus of the Melolonthinae tribe Rhizotrogini (Scarabaeidae) that currently contains 21 species and 2 subspecies, of which 14 species have been recorded from Himalaya region of India. A new species *Panotrogus jagbiri* Kumar, Pandey & Keith, sp. nov. is described from Uttarakhand, India and illustrated. A key to Indian *Panotrogus* species has been prepared.

Keywords: Biodiversity, beetles, new taxa, light trap, taxonomy, Asia, Himalaya, key, Rhizotrogini.

INTRODUCTION

India is a biodiversity rich nation sharing four biodiversity hotspots of the world, i.e., Himalaya, Indo-Burma, Sundaland and Western Ghats (Chandra *et al.*, 2018). The Indian Himalaya biodiversity hotspot is divided into two biogeographic zones *i.e.*, Trans Himalaya and Himalaya, in which Uttarakhand is part of the Himalaya, West Himalaya biotic province (Rodgers, Panwar, & Mathur, 2002). West Himalaya represents the second highest faunal diversity (12022 species/subspecies) after Central Himalaya (14183 species/subspecies) with arthropods representing approximately 86.9% of Indian Himalayan biodiversity. Among the arthropods, the Coleoptera are the most diverse insects with 43% species/subspecies from the Indian Himalaya in 107 families (Chandra & Sidhu, 2009). The Scarabaeidae is a highly diverse family with 10% of the reported species.

Panotrogus Reitter, 1902 (Scarabaeidae, Melolonthinae, Rhizotrogini) is a Palearctic genus of chafers. The species assigned to *Panotrogus* are characterized by antennae with 10 antennomeres, three of which form the antennal club, the shapes of the clypeus and maxillary palps, claws that are cleft at the base, and an aedeagus with slender parameres and a sclerotized inner piece (Keith, 2001, 2002). Currently, it contains 21 species, one of which is divided into two subspecies. Morphologically, it is close to *Pseudopanotrogus* Petrovitz, 1969 from which it differs most clearly by the cleft claws (Keith, 2001, 2002). The genitalia of *Pseudopanotrogus* spp. are usually of special complex structure, while in *Panotrogus* spp. they show a secondary branch.

Recently, MK examined an interesting specimen of *Panotrogus* from Uttarakhand, northwestern India and, after comparing it with all available material and with the literature (mainly Keith, 2001, 2002, 2005, 2009; Kumar *et al.*, 2023), came to the conclusion that it represents an undescribed species that we describe below.

MATERIALS AND METHODS

Study Area

Adult beetles were collected in surveys in the Tehri district of Western Himalaya, Uttarakhand, northwestern India, during the summer of 2021 with the aim to collect undescribed species and determine their distribution patterns. The location of surveys lay between 30.3455°N Latitude and 78.3947°E Longitude (Fig.1). During May to September, when the adult beetles emerge from the soil during dusk and settle on the nearby trees to feed and mate, a mercury vapour lamp light trap (150 W) was run from 7:15 pm to 10:30 pm.

Along with the light trap collections, beetles were also searched for on their host plants with the help of powerful torch light since few species avoid light (Pal, 1977; Prathibha *et al.*, 2018). Beetles were collected from light traps and host plants, were brought into the laboratory where they were killed with ethyl acetate and labelled, pinned and placed in insect cabinets for further identification.

Description of a New Species of *Panotrogus* Reitter, 1902

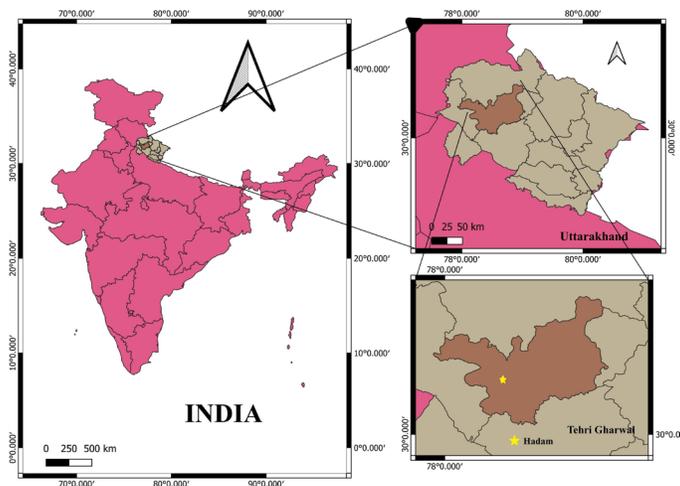


Figure 1. Collection locality of *Panotrogus jagbiri* sp. nov. Yellow star (Hadam).

Specimen preparation

The undescribed *Panotrogus* specimen was collected from light trap, during 2021 from the Hadam village of Chamba locality. Other specimens belonging to the same genus were also examined for species confirmation by comparison with specimens in KDCC and the literature (Keith, 2002, 2005, 2009; Kumar et al, 2023). The abdomen was removed with the help of pointed needle from the specimen's body. The genitalia and speculum gastrale were carefully extracted with forceps from the abdomen and placed in a watch glass under 10% KOH for the removal of muscle and then glued on a pointed card and pinned along with the adult male specimen and label. The external morphological characters of the genitalia were observed through Nikon SMZ745T stereo zoom microscope. Images of the entire beetle were obtained with Nikon D5600 digital camera, while images of the genitalia were compiled using Leica auto montage software. Terminology for the aedeagal structures follows that of D'Hotman and Scholtz (1990). Length was measured from the anterior margin of the clypeus to the apices of the elytra. Data in faunal series of India, zoological records, bulletins, state fauna series, conservation area series, ecosystem series etc., pertaining to *Panotrogus* spp. and its distribution were searched for thoroughly.

RESULTS

Panotrogus jagbiri sp. nov. (Figs 2-3)

Type series: Holotype ♂, India; Uttarakhand, Tehri, Chamba, Hadam, 1587m, 30.3455°N, 78.3947°E, 22.vi.2021, Mayank Kumar leg. (in National Pusa Collection, Delhi, India).

Description: Male: Body 10 mm long; reddish brown, venter partially lighter; covered with equally distant, short, light-coloured setae (Fig. 2).

Clypeus transverse, lateral margins converging towards base, anteriorly angles rounded and margin strongly raised and poorly incised; anterior margin weakly sinuate; punctuation regular medium and condensed in the margins. Clypeo-frontal suture sinuate and clearly visible. Frons with larger punctures than on the clypeus; vertex with dense punctuation bearing setae sloping backwards. Eyes each with a long thick canthus set with brownish setae. Labrum thick; pilosity restricted to lateral parts. Last maxillary palpomere elongated, with a slightly depressed frosted basal area. Antenna with 10 antennomeres, club of three antennomeres and almost equal in length to antennomeres 1-7 combined. (Fig. 2).

Pronotum transverse, widest in the middle, lateral margins converging more clearly anteriorly than posteriorly as an arc of a circle; anterior angles invisible, posterior angles very obtuse not marked; anterior and posterior margins rimmed; lateral margins crenated, with short, brownish, erect setae emerging from between the indentations; surface with uniform punctuation with fine yellow setae directed backwards. Scutellum triangular with disc smooth and punctuation limited to lateral margins.

Elytra elongated, slightly widened posteriorly from the middle; punctuation uniform as on pronotum with uniform backward-tilted small setae; humeral callus strong and punctuated like surrounding integument; apical callus wide, punctuated like humeral callus; epipleural margins not wide under the humerus, tapering at least to the rounded elytral apices, bearing long brownish setae. Presence of micro-pilosity emerging from the punctures as on pronotum. Pygidium triangular, covered with large punctures, long pilosity limited to the apical margins.

Mesosternum and metasternum with dense fine punctuation, bearing long pilosity partially obliterating the integument. Protarsi I, II, III and IV of slightly increasing length with apical inner accessory denticles. Protibiae tridentate on the outer margin, the teeth equidistant, the inner apical spur at the level of middle tooth. All the Claws elongated, slightly curved, with a basal denticle in a neat triangle. Mesofemur with scattered punctures each bearing a very long erect seta. Mesotibia elongated, almost equal in length to the mesofemur, with setose notches in the basal and apical thirds. Metafemur with some punctures scattered on the disc and two piliferous rows bearing long thick erect bristles. Metatibial length about equal to the metafemora with piliferous notches in the apical quarter. Ventral sternites fused in the middle and each segment provided with rows of short bristle located in the middle.

Aedeagus: Endophallus smaller than parameres. Secondary branch of parameres very small, bud like, inserted near the emargination of the parameres (Figs. 3a, b).

Diagnosis: This new species belongs to *the heinzi* group and can be separated from the other species by its secondary bud-like branch and the maxillary palpi which is small and comparatively partially depressed as compared to both *P. heinzi* Keith, 2001 (northern Pakistan and northern Afghanistan) and *P. grossopunctatus* Keith, 2006 (Pakistan).

Etymology: Adjective in the nominative singular. This species is dedicated to MK's grandfather (Jagbir Singh).

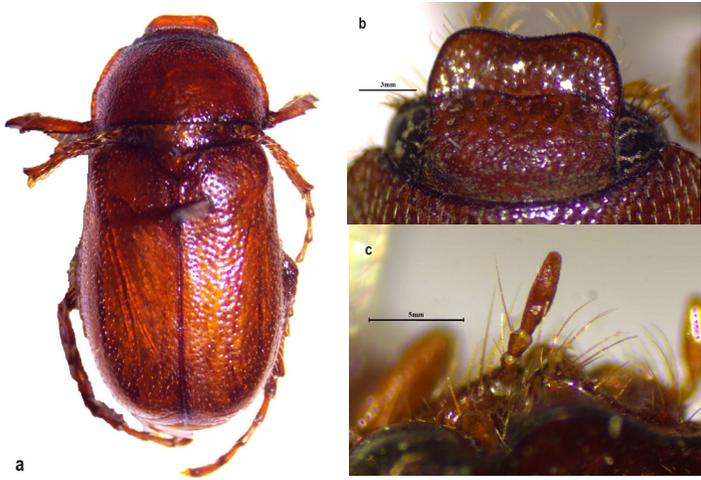


Figure 2. *Panotrogus jagbiri* sp. nov. Holotype. a) adut (male) b) clypeus c) maxillary palp

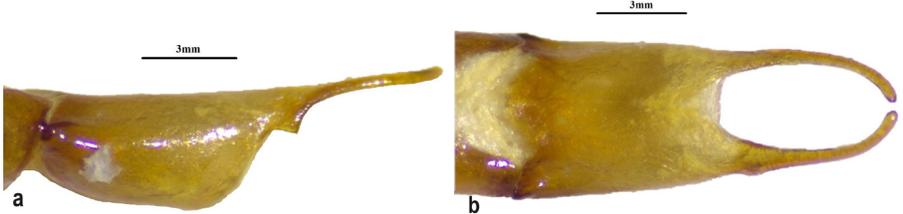


Figure 3. *Panotrogus jagbiri* sp. nov. Holotype. Male parameres: a) lateral, b) ventral.

Key For Indian *Panotrogus* species

- 1. Parameres with convoluted apices, not straight, without secondary branch at base or near middle 2
- 1'. Parameres elongate with a distinct secondary branch at base or near middle 3
- 2. Parameres convoluted into a swan neck with an acicular expansion on their dorsal side. Clypeus with obsolete external angles. Pilosity on pronotal and elytral disc more or less erect and conspicuous *P. hirsutus* Moser, 1913
- 2'. Parameres thickly S-shaped at apex without dorsal acicular expansion. Clypeus with sharp external angles. Pilosity on pronotal and elytral disc very short and inconspicuous *P. schmidti* Keith, 2006
- 3. Secondary branch of parameres very small or minute, triangular, inserted near emargination of parameres; anterior margin of clypeus emarginate 4
- 3'. Secondary branch of parameres thin and distinct, inserted farther 5
- 4. Main branch of parameres thin and elongate, secondary branch of parameres bud like, anterior margin of clypeus weakly sinuate; last joint of maxillary palpi weakly fusiform *P. jagbiri* Kumar, Pandey & Keith, 2023

- 4'. Main branch of parameres evidently flattened near apex, secondary branch of parameres not perpendicular to the axe of parameres, anterior margin of clypeus sinuate; last joint of maxillary palpi fusiform *P. expansus* Keith, 2003
5. Last joint of maxillary palpi fusiform flattened and dull on upper side6
- 5'. Last joint of maxillary palpi fusiform depressed on upper side. Anterior margin of clypeus very weakly emarginate; antennal joint VI not very transverse.....
..... *P. inexpectatus* Keith, 2001
6. Main branch of parameres two times longer than secondary branch, which is directed upwards*P. pakistanus* Keith 2002
- 6'. Main branch of parameres three to four times longer than secondary branch, which is perpendicular *P. batillinus* Bates, 1891

ACKNOWLEDGEMENTS

We thank Mr. Rhaul Uniyal and Sagar for their help in collection of beetles from Hadam and GBPAT Pantnagar for providing necessary facilities during course of investigation.

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First Record of *Leucopholis lepidophora* Blanchard, 1850 (Scarabaeidae; Melolonthinae) from Western Himalayas along with a Checklist of the Genus *Leucopholis* from India

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ABSTRACT

Melolonthinae is a subfamily of June beetles and contains many species of agronomic importance in India. It holds major genera like *Brahmina* (Blanchard 1850), *Eotrichia* (Medvedev 1951), *Holotrichia* (Hope 1837) and *Sophrops* (Fairmaire 1887) in India. A survey based on light trap collection was conducted in the terai foot hills of the Western Himalaya in Uttarakhand to check for the presence of the species. During surveys in the years 2021-22 and 2022-23, various chafer beetles were collected to observe the distribution pattern and their hosts. Among these various species, one beetle species is here reported for the first time i.e., *Leucopholis lepidophora* Blanchard, 1850, from Uttarakhand, India. This also represents their first record for Western Himalaya. Their habitus and male genitalia are provided herein to aid in identification. A preliminary checklist of Indian *Leucopholis* is also provided.

Keywords: June Beetles, Light trap, Himalaya, *Leucopholis* sp. new record, range expansion

Kumar, M. & Pandey, A.K. (2024). First record of *Leucopholis lepidophora* Blanchard, 1850 (Scarabaeidae; Melolonthinae) from Western Himalayas along with a checklist of the genus *Leucopholis* from India. *Journal of the Entomological Research Society*, 26(1), 129-136.

Received: December 15, 2023

Accepted: March 19, 2024

INTRODUCTION

India is a biodiversity-rich nation that shares around 6.4% of the world fauna (Chandra et al, 2018). It has been divided into 27 biogeographic zones, of which the Indian Himalaya is classified into 2 biogeographic zones i.e., Trans Himalaya and Himalaya. Further, these two zones can be classified into seven biotic provinces, of which Uttarakhand comes under the Western Himalayas biotic province (Rodgers, Panwar, & Mathur, 2002). It represents the second highest faunal diversity (12022 species/subspecies) after central Himalayas (14183 species/subspecies), which is overall dominated by arthropods, accounting for approximately 86.9% of Indian Himalaya (Chandra & Sindhu, 2009).

Coleoptera is the largest and most diverse order of class Insecta exhibiting the finest diversity in the world (Kim, Park, Choi, & Park, 2017). In India, the Pleurosticti group is mainly divided into four subfamilies i.e., Melolonthinae, Rutelinae, Dynastinae and Cetoniinae. They commonly belonged to the genera *Holotrichia*, *Brahmina* and *Sophrops*, under the subfamily Melolonthinae (Sreedevi, Tyagi, & Sharma, 2014; Pathania, Chandel, Verma, & Mehta, 2015; Bajad, Dadmal, & Undirwade, 2017; Kumar, Sreedevi, & Singh, 2017).

Leucopholis is a genus under Melolonthinae, which was first erected by Dejean (1833) for the spp. *Melolontha alba* Weber, 1801; *Melolontha stigma* Fabricius, 1798; *Melolontha hypoleuca* Wiedemann, 1819; and *Melolontha rorida* Weber, 1801. *Leucopholis* is characterized by the presence of elytral scales, subdued pronotal serrations, and a prosternal process that is flattened, anteriorly ovoid to spindle-shaped, glabrous. At present, it is distributed mostly in the Oriental region with 56 reported species (Banki et al, 2023). In India, it has been reported from the states of Maharashtra, Karnataka, Kerala, Tamil Nadu, Assam, and Meghalaya as major pest species of areca nut, coconut, sugarcane etc., (Kumar, 1999; Adarash, 2014; Kalleshwaraswamy, Adarsha, Naveena, & Sharanabasappa, 2016; Bhawane & Bhanot 2017; Swamy, Ramasamy, Kalleshwaraswamy, & Adarash, 2019).

Until now, *Leucopholis* species have not been documented from many parts of India, which requires the frequent surveys and monitoring to document and record their species distribution in other parts as well. For the first time in the history of the species biodiversity in the Himalayas, we have recognized the single species of *leucopholis* from the Western Himalayas. Finally, a synopsis and Indian check list of *leucopholis* species are provided, along with images and specimens that are currently available.

MATERIALS AND METHODS

Study Area

Surveys were carried out in the summer of 2021–2023 in Uttarakhand, the Northern state of India, at the foothills of the Western Himalaya, with the goal of collecting adult beetles. Survey locations ranged from 30.06680 N to 79.01930 E (Fig. 1). When the adult beetles emerge from the soil at nightfall and settle on the surrounding trees to

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feed and mate, from May to September of each year, a powerful 150 watt mercury vapour lamp light trap was placed in the aforementioned site between the evening hours of 7: 15 p.m. to 10:30 p.m.

Along with light trap collection, the beetles were also searched in their host plants with the help of a powerful torch light. As beetles were collected from light traps, and were brought into the laboratory, where they were killed with ethyl-acetate and finally labelled, pinned, and placed in an insect cabinet for further identification.

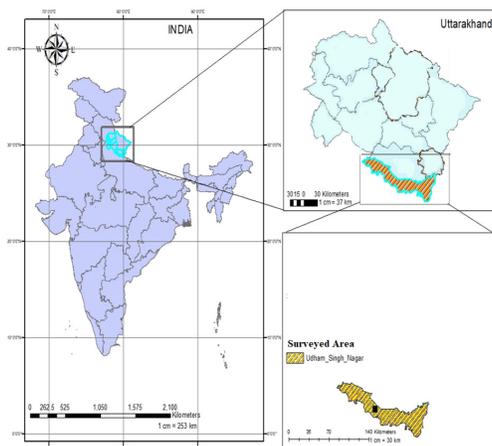


Figure 1. Location of *Leucopholis* sp. collection during 2021-2023.

Specimen Preparation and Microscopy

The *leucopholis* beetle specimens (2) were collected during 2021 to 2023 from the area of Pantnagar locality (29 02' o 60.00" N to 79 30'59.99" E). Samples concerning the departmental museum of Entomology, College of Agriculture, Pantnagar were also examined for species confirmation using several referred papers (Swamy et al, 2019; Adarasha, Kalleshwaraswamy, & Shivanna, 2023). Later, the abdomen was removed with the help of a pointed needle from the specimen's body. Genitalia and speculum gastrale were extracted carefully with forceps from the abdomen, and placed in cavity block under 10% KOH for muscle removal, and finally glued to a pointed card and pinned along with the adult male specimen and label. The external morphological characters of the genitalia of specimens were observed through a nikon SMZ745T stereo zoom microscope and adults' images were obtained with a nikon D5600 digital camera while genitalia image was attached to the microscope using leica auto-montage software. The terminology follows the aedeagus structures by described D'Hotman & Scholtz (1990). Length measurements are from the anterior margin of the clypeus to the apices of the elytra. Data pertaining to *Leucopholis* species and their distribution were searched thoroughly for their documentation and distribution in India (Burmeister, 1855; Sharp, 1876; Brenske, 1892, Brenske, 1894; Barlow, 1899; Dalla Torre, 1912, Sabatinelli, 1993; Thurekar et al, 2012; Swami et al, 2019; Gosh et al, 2022; Adarasha, Kalleshwaraswamy, & Shivanna, 2023).

RESULTS

The present investigation was based on the morphological identification, and structure of male genitalia. The present findings record the first documentation of *Leucopholis lepidophora* Blanchard (1850) from the studied locality (Fig. 1). Results of the survey from sampling sites revealed its first record from the Western Himalaya of the northern part of India.

Leucopholis lepidophora Blanchard 1850 (Figs 2a-b)

Description: Male length 35.0 mm, width 17 mm; female length 36.0 mm, width 17 mm. Body elongate and ovoid; uniformly black or blackish brown. The clypeus anterior margin is wide and strongly convex mesally in males as compared to females without a median cleft, weakly indented and longer than the labrum (Fig. 3a). Labrum punctate with long, fine, stiff reddish orange setae. The mentum is smooth and punctate in the posterior margins. Ocular canthus with small white and large reddish-brown setae. Antennal club length is 2 mm and nearly as long as antennomeres II–VII. Anterior angle of the pronotum rounded and the posterior angle is obtuse and lobed in both sexes. Protibial spurs extend in front of the anterior angle in both sexes. Posterior metatibial end with 19-35 spicules. The prosternal process is dorsally pear-shaped without median depression. Metaventral process length 2.5 mm. Scutellum scales overlap at the anterior angle. Elytral scales are large, less dense (male), sub-ovate shape and off white in colour.



Figure 2. Adults of *L. lepidophora* viz.; a) male (collected specimen), b) female (Museum specimen).

Male: Genitalia length 9.5 mm. Phallobase (5 mm) larger than paramere, sclerotized dorsal anterior margin with horizontal straight mesally and connected with membranous

First Record of Leucopholis lepidophora Blanchard, 1850

tissue. Paramere (4.5 mm) ventral inner posterior margin is inverted, elongate, and hook-shaped; parameres are parallel laterally, apically blunt, and basally pointed (Figs. 4a-c).

Female: Clypeus is weakly convex, weakly recurved, strongly indented laterally, and distinctly narrower than the labrum (Fig. 3b). Antennal club length 1.30 mm, shorter than antennomeres II-VII. Pronotum anterior angle obtuse and posterior angle at right angle. Meta ventral process length 2.3 mm. Posterior metatibia with 35 spicules.

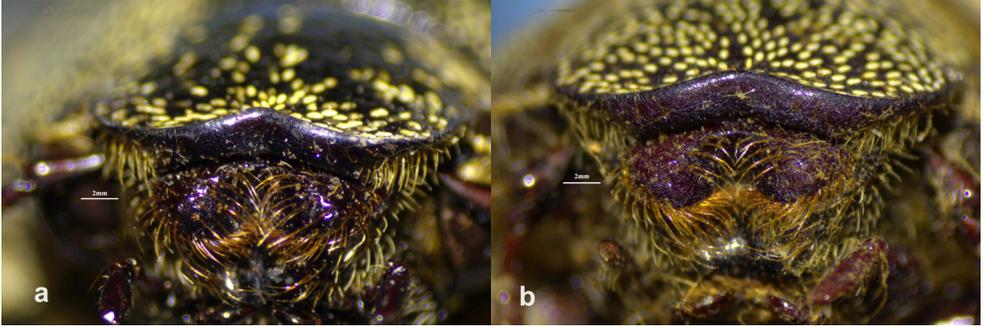


Figure 3. Clypeus of *L. lepidophora*; a) male, b) female.

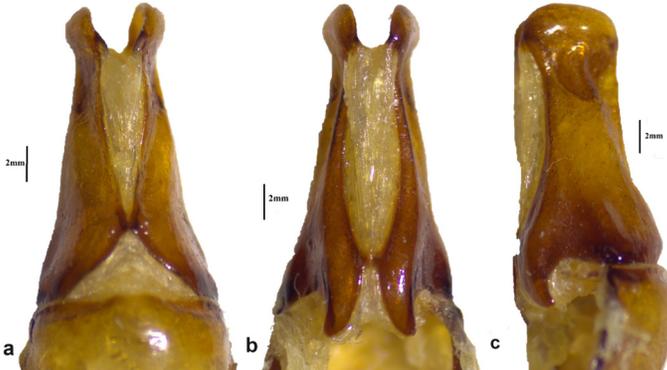


Figure 4. Male genitalia of *L. lepidophora*. Parameres; a) dorsal, b) ventral, c) lateral.

Material examined: India; Uttarakhand, U.S. Nagar, Pantnagar (29 02' o 60.00" N to 79 30'59.99" E), 243m 02.08.2023, 2♂,1♀. U.S. Nagar, Pantnagar, 243m October.1971, 1♀ (Department Museum specimen).

Distribution India: Maharashtra, Karnataka, Kerela, Tamil Nadu, Uttarakhand (New record)

CONCLUSION AND DISCUSSION

The present study of *Leucopholis lepidophora* from the lower foot hills of Western Himalaya recorded its first documentation from the same.

In India, *Leucopholis* species has gained the status of pest in many economical crops like arecanut, sugarcane, cocounut etc (Kumar, 1999; Adarasha, 2014; Kalleshwaraswamy et al, 2016). Usually, *Leucopholis* genus has its three economic important species in India. In those three, *Leucopholis lepidophora* is found in hilly and high-rainfall areas, while *L. coneophora* and *L. burmeisteri* are confined to coastal areas (Swamy et al, 2019). Out of these three species, *L. lepidophora* and *L. burmeisteri* takes two years for completion of life cycles, whereas *L. coneophora* take one year to complete its life cycle (Kumar, 1999). Several studies concerning to their behavioural aspect were also studied which found that *Leucopholis lepidophora* and *L. burmeisteri* did not attract light while *L. coneophora* attract to the light (Veeresh, Vijayendra, Reddy, Rajanna, & Rai, 1982; Jeevan, 2014). At present, due to the unavailability of literature, it demands more studies on the behavioural aspect of *Leucopholis* beetles at various light sources. Another species i.e., *L. crassa* was only documented from the evergreen forest of Brahmaputra, Assam and subtropical pine forest of Meghalaya from north eastern parts of India (Ghosh et al, 2022).

In our present findings, we recorded this species from a terai area of Uttarakhand that comes under broad leaf deciduous forest (Sati & Bandooni, 2018). The table 1 gives an account to current species and their distribution in India. However, at present, their presence is not recorded in many states like Uttar Pradesh, Haryana and Bihar which also contain some portions of the same type of forest. However, Madhya Pradesh, Chhattisgarh and Rajasthan are required to conduct frequent monitoring and surveillance in their agriculture, forest, and conserved areas for the presence of this species. In present findings, its new record from the localities cannot be ignored due to its pest status in other parts of India on many economical crops. Further studies concerning the life cycle would definitely be helpful for documentation of its real impact on crops and other Himalayan states of India.

Table 1: Checklist of *Leucopholis* species from India.

Sl. No.	Species	Distribution	References
1	<i>Leucopholis aberrans</i> Sharp, 1876	India	Sharp, 1876; Dalla Torre, 1912.
2	<i>Leucopholis burmeisteri</i> Brenske, 1894	Karnataka	Burmeister, 1855; Brenske, 1894; Dalla Torre, 1912; Swamy, 2019
3	<i>Leucopholis coenophora</i> Burmeister, 1855	Kerala	Burmeister, 1855; Dalla Torre, 1912; Swamy, 2019
4	<i>Leucopholis crassa</i> Brenske, 1892	Assam, Meghalaya	Brenske, 1892; Dalla Torre, 1912; Sabatinelli, 1992; Gosh et al, 2022.
5	<i>Leucopholis lepidophora</i> Blanchard, 1850	Maharashtra, Karnataka, Kerala, Tamil Nadu, Uttarakhand, (New record)	Blanchard, E. 1850; Dalla Torre 1912; Thurekar et. al. 2012; Swami et. al. 2019; Adarsha, et. al. 2023
6	<i>Leucopholis tetaranus</i> Brenske, 1896	Deccan	Barlow, 1899

ACKNOWLEDGEMENT

We thank the museum curator, Dr. Meena Agnihotri Department of Entomology, College of Agriculture, for providing specimens during the investigation. Thanks to Dr. Devanshu Gupta for providing information on *Leucopholis* genera. Sincerely, thanks to Dr. Ales Bezděk of the Czech Republic and Dr. Orlando A. Calcetas for their generosity in helping us to find scientific papers.

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First record of *Hydroptila ivisa* Malicky, 1972 (Trichoptera, Hydroptilidae) from the Ecoregion 6, Hellenic Western Balkans

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ABSTRACT

Adult caddisfly specimens were collected from the Karadak Mountain in Kosovo along the border area with the Republic of North Macedonia during June 2020. Seven species belonging to seven caddisfly families were found. From this area, we report *Hydroptila ivisa* Malicky, 1972 for the first time for Kosovo and the Ecoregion 6, Hellenic Western Balkans. Range extension for *Synagapetus slavorum* Botosaneanu, 1960, *Hydropsyche emarginata* Navàs, 1923 and *Polycentropus slovenica* Malicky, 1998 is also reported. These findings increase the knowledge on the distribution and diversity of several rare species of caddisflies and especially of the new finding, *H. ivisa* in the Western Balkans and highlight the importance of further studies and conservation efforts for freshwater biodiversity in this region.

Keywords: caddisflies, aquatic insects, Kosovo, biodiversity, rare species, Balkan Peninsula

Bilalli, A., Ibrahim, H., Musliu, M., Geci, D., & Grapci-Kotori, L. (2024). First record of *Hydroptila ivisa* Malicky, 1972 (Trichoptera, Hydroptilidae) from the Ecoregion 6, Hellenic Western Balkans. *Journal of the Entomological Research Society*, 26(1), 137-145.

Received: December 27, 2023

Accepted: March 16, 2024

INTRODUCTION

The knowledge about the caddisflies of the Balkan Peninsula has increased significantly over the past years with the description of several new species and records of other rare species of this order of aquatic insects. In this regard the caddisflies of Kosovo are relatively well investigated (Ibrahimi & Sejdiu, 2018; Ibrahimi & Vehapi, 2017; Ibrahimi, Kučinić, Gashi, & Grapci-Kotori, 2014; Ibrahimi et al, 2014; 2015a, 2015b, 2016a, 2016b, 2018, 2019a, 2019b, 2021, 2023; Ibrahimi, Kuçi, Bilalli, & Gashi, 2017b; Karaouzas et al, 2018; Oláh, Chvojka, Cubuc, Coppa, & Ibrahimi, 2015; Oláh et al, 2018); followed by North Macedonia (e.g., Bilalli, Ibrahimi, & Musliu, 2018; Bilalli et al, 2019; Hinić et al, 2020; Musliu et al, 2020; Oláh, Kovács, & Ibrahimi, 2018; Oláh et al, 2022; Slavevska-Stamenković et al, 2016, 2020, 2021; Valladolid et al, 2022); Albania (Ibrahimi & Bilalli, 2021; Ibrahimi & Kučinić, 2018; Oláh & Beshkov, 2016; Oláh et al, 2022); Serbia (Ibrahimi, Jahiji, & Bilalli, 2017a; Ibrahimi et al, 2022; Oláh et al, 2018, 2019, 2022; Stojanović et al, 2015) and Montenegro (Ibrahimi, Pali, Bilalli, & Musliu, 2019e; Oláh & Beshkov, 2016; Oláh & Kovács, 2014).

Family Hydroptilidae is the largest caddisfly family in terms of species diversity, with 2,642 species found in all faunal regions of the world and distributed in six subfamilies and 76 genera (including three fossil genera) (Thomson, 2023), but the smallest in terms of body size. Adults body size is about 1.5-5 mm (Holzenthal et al, 2007). The Hydroptilidae, known as microcaddisflies, are extremely diverse; larvae occur in a wide array of aquatic habitats, display numerous feeding patterns, and last instars construct a variety of larval cases known collectively for the family as “purse-cases”, exhibiting an interesting hypermetamorphosis observed within Trichoptera only in Hydroptilidae and its sister group, Ptilocolepidae. The Hydroptilidae, are holometabolous insects with a terrestrial adult stage and aquatic larval and pupal stages. The adults are attracted to ultraviolet lights and may congregate in huge numbers at collecting sites, giving them the potential to be one of the most commonly collected families of all Trichoptera. However, the aquatic larvae of Hydroptilidae are often more difficult to collect than the adults (Thomson, 2023).

There is limited available data on the distribution and diversity of hydroptilids in the Balkan Peninsula, including Kosovo. In the past years, some data for the Hydroptilidae family have been reported from Kosovo, such as: Bilalli (2019), Ibrahimi & Sejdiu (2018), Ibrahimi et al, (2012), Ibrahimi (2011), however, more research is needed to fully understand the distribution and diversity of hydroptilids in Kosovo and adjacent areas.

In this paper, we report *Hydroptila ivisa* Malicky, 1972 for the first time from Ecoregion 6, Hellenic Western Balkans and discuss findings of some other rare caddisfly species with limited distribution in the Balkan Peninsula.

MATERIAL AND METHODS

Sampling was carried out at two sampling stations in the Karadak Mountain (Figures 1 & 2). This mountainous area is located in the Hani i Elezit Municipality (near the

border with North Macedonia), between the villages Dimcë and Dërmjak at altitudes up to 600 - 1000 meters a.s.l. The first sampling station (S1) is located at the main stream above the Dimcë village-Dërmjak (N° 42.173038; E° 21.316659; 620 m a.s.l.; Figure 1a). The epifaunal substrate at this sampling station was suboptimal: 40-70% of stream bed and lower banks covered with a mix of substrates favorable for epifaunal colonization and fish cover; embeddedness: gravel, cobble and boulder particles are 20-50% surrounded by fine sediment; sediment deposition: some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools; flow status: water fills >75% of the available channel; or <25% of channel substrate is exposed; bank stability: moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion; bank vegetation protection: 70-90% of the streambank surfaces covered by native vegetation, disruption evident but not affecting full plant growth potential to a great extent; more than one half of the potential plant stubble height remaining; riparian vegetative zone width: width of riparian zone 6-12 meters; human activities have impacted zone a great deal. The second sampling station (S2) is located in the northeastern part of Dërmjak village, specifically at a streamlet known locally as "Te Çeshmja", which is a tributary of the main stream (N° 42.189092 and E° 21.310585, 985 m a.s.l.; Figure 1b). The epifaunal substrate was poor: less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking; embeddedness: gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment; sediment deposition: heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition; flow status: very little water in channel and mostly present as standing pools; bank stability: unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars; bank vegetation protection: less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height; riparian vegetative zone width: width of riparian zone <6 meters: little or no riparian vegetation due to human activities.

Adult caddisfly specimens were collected using ultraviolet light traps (UV), entomological nets (EN), and hand-picking. Sampling was carried out during June 2020. Collected specimens were preserved in 80% ethanol. The specimens were identified under a stereomicroscope with determination keys from Malicky (2004) and Kumanski (1985, 1988). Systematic nomenclature follows Morse (2024).

The collected material is deposited at the Laboratory of Zoology of the Faculty of Mathematics and Natural Sciences, University of Prishtina, Kosovo



Figure 1. Sampling stations in the Karadak Mountain, Kosovo. a) S1 - mainstream, b) S2 - “Te Çeshmja”.



Figure 2. Map of sampling stations in the Karadak Mountain, Kosovo.

RESULT AND DISCUSSION

During this investigation we found seven species belonging to seven families of caddisflies (Rhyacophilidae, Glossosomatidae, Hydroptilidae, Hydropsychidae, Polycentropodidae, Limnephilidae, and Beraeidae). All families were represented with a single species each. The highest number of specimens belongs to the species *Polycentropus slovenica* (8 males, 1 female), and *Hydropsyche emarginata* (5 males, 2 females), while *Ernodes articularis* was found with a single specimen.

Species list

Family: RHYACOPHILIDAE Stephens, 1836

Genus: *Rhyacophila* Pictet, 1834

***Rhyacophila loxias* Schmid, 1970**

Material examined: Kosovo, Dërmjak, (UV) 28.06.2020, 2 ♂♂, leg.: Bilalli. A., Musliu., M., and Geci, D.

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Family: GLOSSOSOMATIDAE Wallengren, 1891

Genus: *Synagapetus* McLachlan 187

***Synagapetus slavorum* Botosaneanu, 1960**

Material examined: Kosovo, Dërmjak, (UV) 28.06.2020, 4 ♂♂, leg.: Bilalli. A., Musliu., M., and Geci, D.

Family: HYDROPTILIDAE Stephens, 1836

Genus: *Hydroptila* Dalman, 1819

***Hydroptila ivisa* Malicky, 1972 ***

Material examined: Kosovo, Dërmjak, (UV) 28.06.2020, 3 ♂♂, leg.: Bilalli. A., Musliu., M., and Geci, D.

Family: HYDROPSYCHIDAE Curtis, 1835

Genus: *Hydropsyche* Pictet, 1834

***Hydropsyche emarginata* Navàs, 1923**

Material examined: Kosovo, Dërmjak, (UV) 28.06.2020, 5 ♂♂, 2 ♀♀, leg.: Bilalli. A., Musliu., M., and Geci, D.

Family: POLYCENTROPODIDAE Ulmer, 1903

Genus: *Polycentropus* Curtis, 1835

***Polycentropus slovenica* Malicky, 1998**

Material examined: Kosovo, Dërmjak, (UV) 28.06.2020, 8 ♂♂, 1 ♀, leg.: Bilalli. A., Musliu., M., and Geci, D.

Family: LIMNEPHILIDAE Kolenati, 1848

Genus: *Micropterna* Stein, 1873

***Micropterna lateralis* (Stephens, 1837)**

Material examined: Kosovo, Dërmjak, (UV) 28.06.2020, 3 ♂♂, 2 ♀♀, leg.: Bilalli. A., Musliu., M., and Geci, D.

Family: BERAIDAE Wallengren, 1891

Genus: *Ernodes*, Wallengren, 1891

***Ernodes articularis* (Pictet, 1834)**

Material examined: Kosovo, Dërmjak, (Te Çeshmja): (EN) 28.06.2020, 1 ♂, leg.: Musliu. M. Bilalli. A., and Geci, D.

Hydroptila ivisa was described from Austria and is also found in a limited number of localities in Bulgaria, Czech Republic, France, Germany, Italy, Slovenia, and Ukraine (Thomson, 2023). Despite intensive caddisfly investigations during the past years in Kosovo and neighboring countries (Ibrahimi, Pali, Bilalli, & Musliu, 2019e; Ibrahimi, Jahiji, & Bilalli, 2017a; Ibrahimi et al, 2018; 2019c, 2019d; 2021; 2023; Karaouzas et al, 2018; Oláh & Kovács, 2014; Oláh, Chvojka, Cubuc, Coppa, & Ibrahimi, 2015; Oláh & Beshkov, 2016; Oláh et al, 2018a, 2019; 2022, Valladolid et al, 2022), this

species has never been found, suggesting a limited distribution and low populations. The species is also known from single localities in Italy and Bulgaria. In Italy, *H. ivisa* was reported from rhithral stream zones only (Corallini et al, 2013) which is also the case with the locality in the Dermjak stream in Kosovo where this species was found.

During this investigation, we encountered adult specimens of *H. ivisa* during June. Consistent with our findings, previous research by Komzák & Kroča (2011) also reported this species during the same month.

This discovery of this rare species of the Hydroptilidae family is an important contribution to the knowledge of aquatic biodiversity, considering the lack of knowledge about this family in the Balkan Peninsula. The finding of *Hydroptila ivisa* in new locations and for the first time in the Ecoregion Hellenic Western Balkans suggests a wider distribution of this species than previously known. This highlights the need for further research on the distribution, abundance, and ecology of this species.

During this investigation, we found several other species with limited distribution in the Balkan Peninsula, such as *Polycentropus slovenica*, *Synagapetus slavorum* and *Hydropsyche emarginata*.

Polycentropus slovenica, initially discovered in Slovenia, is also found in Italy and Bosnia and Herzegovina. It was first described as subspecies of *Polycentropus ierapetra*, but was later elevated to species level (Oláh et al, 2022). In 2014, it was reported for the first time from Kosovo (Ibrahimi, Kučinić, Gashi, & Grapci-Kotori, 2014) and was afterwards found at various locations in the Bjeshkët e Nemuna and Karadak Mountain (Ibrahimi et al, 2019c). Our current finding extends the species' range and most probably future investigations will show that this species is even widely distributed in the Balkan Peninsula, unlike all other species of the *Potamophylax ierapetra* complex which are narrow endemics of certain areas.

Range extension for *Synagapetus slavorum* and *Hydropsyche emarginata* is also reported in this work. Both species are scarcely distributed in the Balkan Peninsula (Neu et al, 2019) and are known from only few localities in Kosovo (Ibrahimi et al, 2012). Thus, their finding improves greatly the knowledge on the distributional patterns for these rare species.

This investigation contributes to the knowledge on the distribution and diversity of the Trichoptera and especially of the Hydroptilidae family in the Balkan Peninsula. Additionally, the data gathered in this study can be used to improve the understanding of the ecological roles and interactions of hydroptilids in freshwater ecosystems.

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Taxonomic Notes on the Soldier Fly Genus *Odontomyia* Meigen, 1803 (Diptera: Stratiomyidae) from Pakistan with a New Country Record

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ABSTRACT

The soldier fly genus *Odontomyia* Meigen, 1803 (Diptera: Stratiomyidae) is revised from Pakistan. Two species are re-described and illustrated: *Odontomyia ochropa* Thomson, 1869 and *Odontomyia solennis* Walker, 1851. Of these, *Odontomyia solennis* is newly recorded for Pakistan. An illustrated key and a distributional map of these two species in Pakistan are provided.

Keywords: Oriental Region, re-description, morphology, Northern Area.

Hassan, M.A., Shehbaz, M.H., Shehzad, A., Maryam, Z., Khatak, N., & Bodlah, I. (2024). Taxonomic notes on the soldier fly genus *Odontomyia* Meigen, 1803 (Diptera: Stratiomyidae) from Pakistan with a new country record. *Journal of the Entomological Research Society*, 26(1), 147-156.

Received: January 26, 2024

Accepted: March 14, 2024

INTRODUCTION

The genus *Odontomyia* Meigen, 1803, belongs to the subfamily Stratiomyinae (Diptera: Stratiomyidae) and is one of the most species-rich genera in the tribe Stratiomyini, with approximately 219 species distributed worldwide, except Antarctica: Afrotropical (46), Australian (59), Nearctic (30), Neotropical (25), Oriental (27) and Palearctic realms (41) (Woodley, 2001; 2011; Wang, Perng, & Ueng, 2007; Rozkošný & Woodley, 2010; Yang, Zhang, & Li, 2014; Hauser, Woodley, & Fachin, 2017; 2022; Yang & Yang, 2021). To date, 10 species of the genus *Odontomyia* have been recorded from Indonesia, nine from India, three from Sri Lanka, two from Philippines and Malaysia, and one species each from Myanmar, Japan, Singapore, and Pakistan within the Oriental region (Woodley, 2001; Hassan, Bodlah, Iqbal, & Jabeen, 2017). So far, only 12 species under seven genera in four subfamilies: Clitellariinae (1 genus, 1 species), Hermetiinae (1 genus, 1 species), Sarginae (3 genera, 6 species), and Stratiomyinae (3 genera, 5 species), and have been recorded from Pakistan (Woodley, 2001; Hassan et al, 2017; Hassan, Bodlah, Malik, & Gull-E-Fareen, 2019).

In this study, we revised the genus *Odontomyia* Meigen, 1803 from Pakistan and recorded *Odontomyia solennis* Walker, 1851 as a new addition to the stratiomyid fauna of the country, thus recorded two species of the genus *Odontomyia* from Pakistan.

MATERIAL AND METHODS

The adult specimens were collected during 2016 to 2019 by means of sweep-netting in the Himalayan regions of Pakistan. The taxonomic study was conducted using a Nikon SMZ 745 stereomicroscope and images for adult habitus were taken with a Nikon D800 digital camera with a Nikon MICRO NIKKOR 105 mm lens, while the genital photographs were taken with a Canon 7D Mark II digital camera attached with a Nikon SMZ18 microscope. The species were identified by using the following available source of literature: Brunetti (1920, 1923), Nerudová-Horsáková, Kovac, & Rozkosný (2007), and Hassan et al (2017), and compared with the available type photographs of *Odontomyia solennis* Walker. Species distribution map is created using ArcGIS 10.4.1 software. The studied specimens are deposited at National Insect Museum Islamabad, Pakistan (NIM).

RESULTS

Taxonomy

Family Stratiomyidae

Subfamily Stratiomyinae Latreille, 1802

Genus *Odontomyia* Meigen, 1803

Eulalia Meigen, 1800: 21. Type species: *Musca hydroleon* Linnaeus, 1758 (by designation of Coquillett, 1910: 541). Suppressed by ICZN, 1963: 339.

Odontomyia Meigen, 1803: 265. Type species: *Musca hydroleon* Linnaeus, 1758 (by designation of Westwood, 1840: 130).

Taxonomic Notes on the Soldier Fly Genus Odontomyia

Trichacrostyilia Enderlein, 1914: 607. Type species: *Stratiomys angulata* Panzer, 1978. Original designation.

Orthogoniocera Lindner, 1951: 187. Type species: *Odontomyia hirayamae* Matsumura, 1916. Monotypy.

Cyrtopus Bigot, 1883: 132. Type species: *Cyrtopus fastuosus* Bigot, 1883. Monotypy. Synonymized by Rozkošný & Weedley, 2010: 291.

Scapanocnema Enderlein, 1914: 610. Type species: *Scapanocnema spathulipes* Enderlein, 1914 (by original designation). Synonymized by Rozkošný & Weedley, 2010: 291.

Diagnostic note

Adult *Odontomyia* species exhibit a remarkable morphological diversity, including a large variety of color pattern and sizes, but can be distinguished by the following characters: head with eyes bare or pilose, contiguous in males, widely separated in females; antennae with scape and pedicel nearly subequal, ratio of scape to pedicel usually not more than 1.5: 1; flagellum with six flagellomeres, last two flagellomeres forming a short stylus; scutellum with a distinct pair of lateral spines; wing vein R2+3 present; veins M1 and M2 present, M3 vestigial or absent; vein M4 arising from M-cell (Yang 1995; Wang et al, 2007; Nerudová-Horsáková et al, 2007; Rozkošný & Woodley 2010; Rozkošný & Kovac 2014; Hauser et al, 2017).

Key to species of the genus *Odontomyia* from Pakistan

1. Face and frons yellow in both male and female (Fig. 1c-d); scutellum yellow, slightly black at base (Fig. 1a-b) *O. ochropa* Thomson
 - Face and frons black, except mouth edge yellow (Fig. 4e, f), frons in female with a pair of dark brown markings above antennae (Figs 4e, f); scutellum black, slightly yellow at apex (Figs 4a, b) *O. solennis* Walker

Odontomyia ochropa Thomson, 1869

(Figs 1a-h, 2a-h, 6)

Odontomyia ochropa Thomson, 1869: 456. Type locality: Philippines (Luzon: Manila).

Odontomyia dorsoangulata Brunetti, 1920: 68. Type locality: India. Synonymized by Nerudová-Horsáková et al, 2007: 112.

Distribution: **Oriental.** **Thailand:** Muak Lek, Bangkhen, Lang Suan; **India:** Bihar, Karnataka (Bengaluru), Tamil Nadu, West Bengal (Sundarban); **Pakistan:** Punjab, Shakargarh; **Philippines:** Luzon, Manila; **Singapore:** Lim Chu Kang; **Australian:** USA (Hawaii) (Brunetti, 1920; Majumder & Parui, 2001; Woodley, 2001; Nerudová-Horsáková et al, 2017; Hassan et al, 2017; Roy, Chakraborty, Parui, & Mitra, 2018).

Material examined. Pakistan: Gilgit-Baltistan, Baltistan Division, District Shigar, Hashu pi Bagh, 22.07.2017, 1♀ leg M.A. Hassan (NIM), District Skardu, Kresmathang Olding, 12.07.2019, 1♂ 1♀, leg M.A. Hassan (NIM).

Re-description: Male. Body length (excluding antennae): 12 mm; wing length: 8.5 mm.

Head rounded, slightly wider than thorax in dorsal view (Fig. 1a, b). Ocellar triangular prominent, shining black, ocelli brownish yellow. Eyes bare, dark brown to black. Compound eyes contiguous for a distance equal to between the upper frons and anterior ocellus. Face, frons, and vertex reddish yellow to pale brown. Antenna (Fig. 1c, d) ochre yellow, brownish at apex; scape longer than pedicel; flagellum with six flagellomeres, each flagellomeres longer than wide, last two flagellomeres forming a short stylus. Face with a distinct median tubercle, covered with short pale hairs on either sides, middle part almost bare. Ventral parts of head and gena yellow.

Thorax black (Fig. 1a, b), but postpronotal callus, scutellum and scutellar spines yellow; scutum black, densely punctate and finely pale, whitish to golden haired, lateral marginal hairs longer than in center; scutellum yellow but its narrow base at lateral margins black. Pleura yellow, except on upper part of propleura above fore coxa, broad upper and posterior margin of anepisternum, posterior corner of katepisternum, outer part of laterotergite, and lower part blackish, covered with densely and finely pale, whitish to golden haired (Fig. 1g, h). Legs yellow, except basal part of fore and hind coxa slightly blackish, covered with short pale hairs. Wing membrane hyaline, veins and pterostigma pale yellow. Vein M3 virtually absent, its short basal part being barely visible as an indistinct fold. Thoracic squama yellowish, with long marginal fringe. Halter pale brown, knob pale yellow.

Abdomen: Tergites yellow with a median triangular to broad blackish markings. Abdominal pile pale, mostly appressed, longer and erect only on anterolateral parts. Sternites yellow, and bare (Fig. 1a, b).

Terminalia: Epandrium subquadrate, with both proximal corners pointed; epiproct subtriangular, covered with short yellow hairs; cerci relatively short, subquadrate, slightly narrow at base, covered with short yellow hairs; synstenites subtrapezodial, with a pair of distinct medial process; gonostylus curved inwards and pointed apically; phallic complex tripartite (Fig. 2a-g).

Female. Body length (excluding antennae): 9.5 mm; wing length: 7.2 mm. Similar to male, except the following characters. *Head:* Eyes broadly dicoptic; face, frons, vertex, occiput, and gena yellow; ocellar triangle black, with a distinct fine dark median groove between anterior ocellus and base of antennae; frons wide, slightly narrower than face, margins parallel-sided in dorsal view; occiput wide, distinctly visible in lateral view. *Thorax:* Scutellum yellow, except proximal 1/3 blackish. *Abdomen:* Tergites yellow, black markings usually broader than in male and sometimes with regular or even irregular small pale spots in the dark areas (Fig. 1b).

Taxonomic Notes on the Soldier Fly Genus Odontomyia

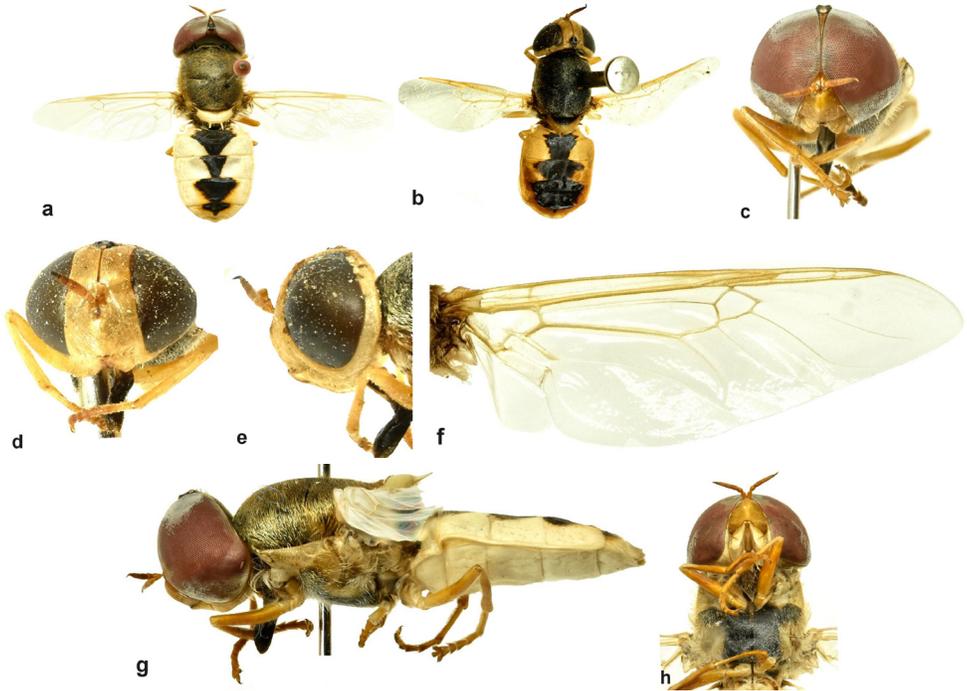


Figure 1. *Odontomyia ochropa* Thomson. a) male dorsal habitus; b) female dorsal habitus; c) male frontal view; d) female frontal view; e) female head in lateral view; f) wing; g) male lateral view; h) male ventral view.

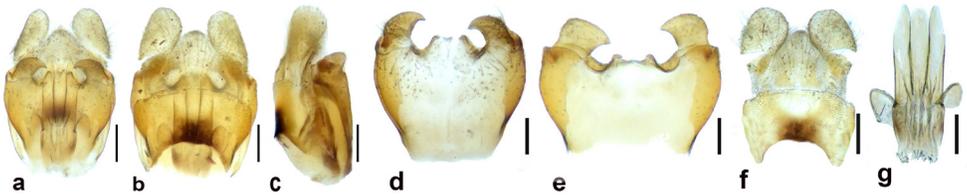


Figure 2. *Odontomyia ochropa* Thomson. Male genitalia: a) ventral habitus; b) dorsal habitus; c) lateral habitus; d) synsternum, dorsal habitus; e) synsternum, ventral habitus; f) epandrium, dorsal view; g) phallic complex.

***Odontomyia solennis* Walker, 1851**

(Figs 3a-e, 4a-h, 5a-g, 6)

Odontomyia solennis Walker, 1851: 79. Type locality: India.

Distribution: **India:** Bihar, Odisha, Uttar Pradesh, West Bengal (Brunetti, 1920; Woodley, 2001). **Pakistan:** New country record (present study).

Material examined. Pakistan: Azad Kashmir, Poonch division, District Poonch, Banjosa Lake, 14.04.2016, 1♀, leg M.A. Hassan (NIM), 20.04.2016, 1♂ 1♀, leg M.A. Hassan (NIM), 23.04.2016, 1♂, leg M.A. Hassan (NIM).

Re-description. Male. Body length (excluding antennae): 11.2 mm; wing length: 8.5-8.8 mm.

Head: rounded, slightly wider than thorax in dorsal view (Fig. 3a, b). Ocellar triangular prominent, shining black, ocelli brownish yellow. Occiput indistinct. Eyes bare, dark brown to black. Compound eyes contiguous for a distance equal to between the upper frons and anterior ocellus. Face, frons, and vertex black. Antenna (Fig. 3a-b) dark brown to brownish yellow; scape and pedicel subequal in length; flagellum with six flagellomeres, first four flagellomeres punctuated, last two flagellomeres forming a short stylus. Face with a distinct median tubercle, covered with short pale hairs on either sides, middle part almost bare (Fig. 3b). Area around the mouth yellow. Gena black, covered with short pale hairs (Fig. 3d).

Thorax: black (Figs. 3a, d, 4a-d), but hind margin of scutellum narrowly yellow, scutellar spines yellow or green with dark apex; scutum black, finely covered with short whitish to golden haired, lateral marginal hairs longer than in center. Pleura black, covered with whitish to golden haired (Figs. 3d, 4d). Legs yellow, except basal part of fore and hind coxa slightly blackish, covered with short pale hairs. Tarsi brownish, fore and mid tarsi slightly brownish, hind tarsi dark brown towards apex (Figs. 3d-e, 4c-d). Wing membrane hyaline, veins and pterostigma brownish yellow. Vein M3 virtually absent, vein M1 indistinct at proximal half. Thoracic squama yellowish, with long marginal fringe. Halter dark brown, knob green or pale yellow (Fig. 3a).

Abdomen: Tergites green to brownish yellow, with a narrow median longitudinal blackish marking on tergites 1-3. Abdominal pile pale, indistinct, mostly appressed. Sternites greenish to pale yellow, with indistinct pale hairs (Fig. 3e).

Terminalia: Epandrium subquadrate, with both proximal corners pointed; epiproct subtriangular, covered with short yellow hairs; cerci relatively short, subquadrate, slightly narrow at base, covered with short yellow hairs (Fig. 5f); synstenites subtrapezoidal, with a median rounded process slightly invaginated in center; gonostylus rounded, curved inwards and pointed apically (Fig. 5d-e); phallic complex tripartite (Fig. 5g).

Female. Body length (excluding antennae): 9.0-10.5 mm; wing length: 8.0-8.3 mm. Similar to male, except the following characters: *Head:* Eyes broadly dichoptic; face, frons, vertex, occiput, and gena black; ocellar triangle black, ocelli dark brown; frons wide, slightly narrower than face, with a pair of median dark brown markings above antennae, lateral margins parallel-sided in dorsal view, covered with short pale and golden hairs; face black, with the area around the mouth yellow, gradually widening at lateral margins in lower half, densely covered with pale hairs, more prominent at lower half in lateral margins; occiput indistinct. *Thorax* black (Figs. 3a, 4a-b), but hind margin of scutellum narrowly yellow, scutellar spines yellow or green with dark apex; scutum black, finely covered with short whitish to golden haired, lateral marginal hairs longer than in center. *Abdomen* (Figs. 3a, 4a-b): Tergites 1 and 2 green to brownish yellow, tergites 3-5 mostly black, but the lateral margins of tergites 3 and 4, and hind margin of tergite 5 green to brownish yellow. Abdominal pile pale, indistinct, mostly appressed. Sternites green to brownish yellow, with indistinct pale hairs (Fig. 3e).

Taxonomic Notes on the Soldier Fly Genus Odontomyia

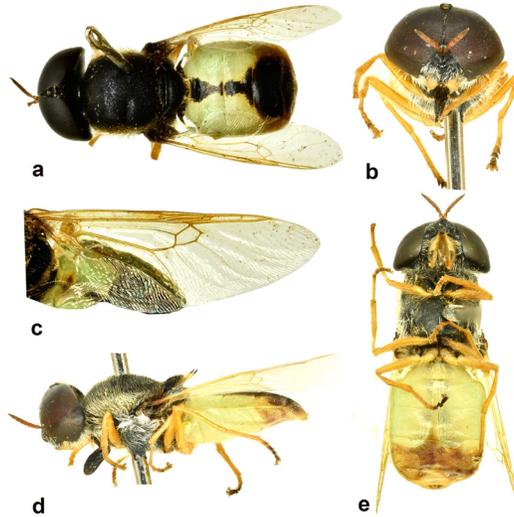


Figure 3. *Odontomyia solennis* Walker. Male: a) dorsal habitus; b) frontal view; c) wing; d) male lateral view; e) ventral view.

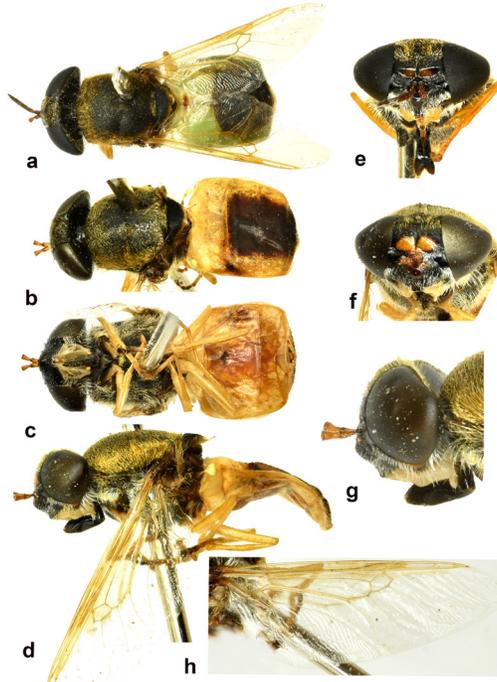


Figure 4. *Odontomyia solennis* Walker. Female: a-b) dorsal habitus; c) ventral view; d) lateral view; e-f) head in frontal view; g) head in lateral view; h) wing.



Figure 5. *Odontomyia solennis* Walker. Male genitalia: a) ventral habitus; b) dorsal habitus; c) lateral habitus; d) synsternum, dorsal habitus; e) synsternum, ventral habitus; f) epandrium, dorsal view; g) phallic complex.

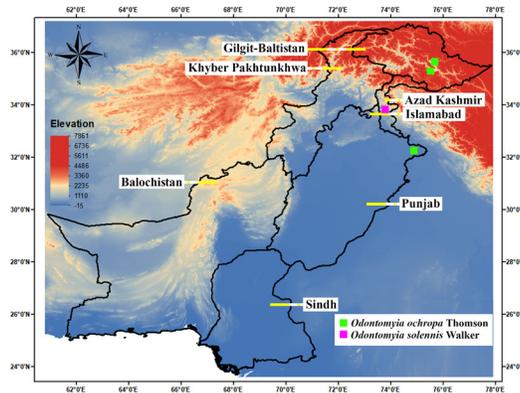


Figure 6. Distribution map of *Odontomyia* species in Pakistan.

DISCUSSION

The taxonomic history of the Pakistani soldier flies dates back to 1920 when Brunetti described *Oxycera albomicans* Brunetti, 1920 and *Stratiomyia fulvescens* Brunetti, 1920 from Abbottabad and Peshawar Districts of the Khyber Pakhtunkhwa province, respectively, in the present territory of Pakistan. Thereafter, in the world catalog of the Stratiomyidae, Woodley (2001) provided the distribution of seven species in Pakistan: *Adoxomyia heminopla* (Wiedemann, 1819), *Oxycera albomicans* Brunetti, 1920, *Ptecticus melanurus* (Walker, 1848), *Sargus flaviventris* Wiedemann, 1824, *Sargus gemmifer* Walker, 1849, *Sargus mactans* Walker, 1859, and *Stratiomyia fulvescens* Brunetti, 1920. The soldier fly of Pakistan has received less attention, with only a few papers have been published in recent years. Recently, Hassan et al (2017) and Hassan et al (2019) added five new additions to the soldier fly fauna of Pakistan: *Odontomyia ochropa* Thomson, 1869, *Oplodontha minuta* (Fabricius, 1794), *Oplodontha rubrithorax* (Macquart, 1838), *Ptecticus kerteszi* De Meijere, 1924, and *Ptecticus vulpianus* (Enderlein, 1914). At present, the stratiomyid fauna of Pakistan has not yet been thoroughly studied, as many areas are still unexplored and need comprehensive investigations in the future studies. This study is one in a series on the taxonomic studies on the stratiomyid fauna of Pakistan aiming to present a review on the genus *Odontomyia* with a new record from Pakistan.

ACKNOWLEDGMENTS

We are grateful to Dr. Martin Hauser (Plant Pest Diagnostics Branch, Department of Food & Agriculture, California, USA) for providing valuable insights into the diverse morphology and taxonomic identification of Asian *Odontomyia* species.

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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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