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Mating Behaviour and Egg Laying Pattern of the Areca Nut White Grub, *Leucopholis lepidophora* (Coleoptera: Scarabaeidae)

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ABSTRACT

The mating behaviour and egg laying pattern of the areca nut white grub, *Leucopholis lepidophora*, were studied in the Shivamogga district's Hosanagara taluk. Females were active between 18:40 and 19:20 hrs, when light intensity dropped to 83.62 lux. The most males approached the virgin female at 18:50 hrs (15.94 lux) and the session ended at 19:20 hrs (0.26 lux). The typical mating behaviour of *L. lepidophora* was investigated. Female beetles began abdominal expansion and contraction, which could indicate that semiochemical secretion has begun. Males then approached, calling out to females. Male raised all of its legs, turned its body ventrally, and became suspended by the genitalia after inserting genitalia into the female abdomen. Copulation lasted an average of 74.60±6.61 minutes. The adult female's pre-oviposition period, oviposition period, and postoviposition period were 9.56± 2.04 days, 3.72± 2.35 days, and 5.24± 4.67 days, respectively. Female longevity ranged from 11 to 28 days, with an average of 18.52± 4.91 days. The incubation period was 9.56± 2.04 days, with varying fecundity and clutch intervals ranging from 24-48 hours. Experiment on egg laying pattern revealed that females waited an average of 9.56± 2.04 days before oviposition began, after which they began to lay varying numbers of eggs at an average clutch interval of approximately 24-48 hours.

Keywords: Lux, semiochemical, Copulation, fecundity, virgin females.

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INTRODUCTION

White grubs are the severe arecanut pest in India. Many species of white grubs are notorious pests of many cultivated crops in India. The arecanut root grubs belong to the Melolonthinae subfamily, and the genus *Leucopholis* Dejean, (Coleoptera: Scarabaeidae: Melolonthinae) is an important pest species feeding on roots of areca nut in malnad and coastal belts of Karnataka (Adarsha, 2014; Kalleshwaraswamy, Adarsha, Naveena, & Sharanabasappa, 2015). The genus *Leucopholis* were successfully identified by male genital structures such as aedeagus and endophallus differed significantly between three species namely *L. lepidophora* Blanchard, *L. burmeisteri* Brenske and *L. coneophora* Burmeister. They were successfully identified by employing COXI gene (Mahadeva Swamya, Asokan, Kalleshwaraswamya, & Adarsha, 2019). Grubs of these *Leucopholis* beetles cause damage to the roots by feeding resulting in symptoms like yellowing of leaves, stem tapering, and nut fall ultimately leading reduced vigour, yield loss and death of palm (Kumar, 1999). Affected palms loose anchorage due to loss of roots and topple down when disturbed (Nair & Daniel, 1982). Six to eight grubs are enough to kill palms (Prakash, Kumar, & Ravikumar, 2011). Severe damage and yield loss noticed in paddy field converted areca nut gardens because of sandy loam soil (Adarsha, 2014). Kalleshwaraswamy, et al, 2015 reported that there was 27.86-36.97 per cent damage by *L. lepidophora* with a yield reduction of 39.79-41.60 per cent in different districts of hilly regions of Karnataka. *L. burmeisteri*, found to be restricted to the coastal area and reported to cause 28.80 per cent damage with a yield reduction of 39.79-41.16 per cent.

The early instar larvae generally feed on the humus for survival (Veeresh, 1977) which may not call for closer association with the host plants. Later instars are near to the root zone and feed on the roots of the areca nut (Veeresh, 1977; Kumar, 1999; Adarsha, 2014). Several integrated management strategies have been adopted to tackle the menace of different species of areca white grubs (Veeresh, Vijayendra, Reddy, & Rajanna, 1982; Kumar, 1999). This includes larval collection by digging soil, soil application of biocontrol agents and insecticides, flooding with insecticidal use and adult collection during their emergence (Veeresh et al, 1982; Kumar, Prakash, Belavadi, & Chandrashekara, 2011; Prabhu, Rakesha, & Balikai, 2011; Adarsha, Kalleshwaraswamy, Pavithra, & Sharanabasappa, 2015b; Adarsha, Shivanna, & Kalleshwaraswamy, 2017; Kalleshwaraswamy, Adarsha, Naveena, & Kumar, 2017; Adarsha, Pavithra, Kalleshwaraswamy, & Kavita Hegde, 2018). Generally, control of soil-dwelling pests of areca nut through insecticide application not exposed to direct contact of insects with the chemicals and other soil properties may hinder the effectiveness of the chemicals (Arakaki et al., 2004b). Chemical applications to soil may create an adverse effect on soil arthropods, earthworms and other natural enemies as well (Adarsha, Kalleshwaraswamy, & Pavithra, 2015a) and also contaminate the groundwater (Fukaya et al, 2009). Hence, there is a need to develop eco-friendly and environmentally safe management practices.

Adults of *L. lepidophora* emerge from June to October from the ground to mate in the evening (Kumar, 1999; Chanakeshava, 2006; Adarsha, 2014; Kalleshwaraswamy, Adarsha, Naveena, & Sharanabasappa, 2016). The previous studies have indicated that a female beetle secretes sex pheromone to which the male beetles are attracted. Once emerged, the males are attracted to the pheromone and hover around the female. By this, an innovative idea of a female baited trap was designed to attract and collection of male beetles. Synthetic sex pheromones have the potential for monitoring and control but also the advances knowledge of *L. lepidophora* mating behaviour, and egg laying pattern is required. Hence, this study is planned to find out information on the mating behaviour of areca nut white grub beetles.

MATERIALS AND METHODS

Experimental site

The study conducted in white grub infested areca nut garden at Gulukoppa (Hosanagara taluk, Shivamogga district; 13°52´ N; 75°12´ E, 692 msl). Twenty-year-old gardens with a white grub incidence of about 50 per cent selected for the experiment.

Calling time and male attraction by virgin females

Virgin females placed in a bucket trap with a small plastic box. These traps were individually tied on the stems of areca nut at 5 feet height and 10 m distance. The virgin females were set at 18:30 hours. This experiment repeated for five times in different days to get more accuracy. Calling posture and attraction of male beetles were recorded at every 10 minutes interval. All males that approached the females were caught in the bucket trap, every 10 min count of the males were taken. Light intensity was measured at 5 feet above the ground for every 10 min from 18:30 to 19:30 hrs. Light intensity measured using a digital lux meter (B. S. K. Technologies, EquinoxTM Model number: EQ-1301).

Mating behaviour and copulation duration

Field observations made during the emergence period immediately after dusk, i.e., between 18:30 to 21:00 hrs by visually searching throughout the arecanut garden. Mating behaviour and copulation duration of 15 newly emerged *L. lepidophora* were observed in areca nut garden at different time intervals. From 18:30 to 21:00, every 15 min intervals pairs in mating were visually surveyed with a head torch. The time of start and ending of copulation were recorded.

Egg laying pattern of the field-collected mated female of *L. lepidophora*

A laboratory experiment was conducted by using field mated beetles. Freshly mated females were collected from the field and then transferred to the laboratory for further studies. Field collected mating pairs were placed individually in plastic containers (17cm ht. X 13 cm dia.) for egg laying. These plastic containers were filled with moist sandy loam soil which was previously taken from the white grub infested

field. The adults collected were maintained until the females die. Pre-oviposition, Oviposition, post-oviposition fecundity and longevity of female beetle were recorded. Observations were taken daily and clutch sizes, inter-clutch interval and eggs per clutch were recorded.

RESULT

Calling and male attraction by virgin females

Virgin females activity started at around 18:40 hrs when light intensity fell to 83.62 lux (Table 1). This considered as a calling period of adult female beetles. Calling behaviour of virgin female beetles increased at 18:50 hrs and light intensity was 15.94 lux, but it was ceased at 19:20 hrs (0.26 lux). Males are started to approach calling virgin females at 18:40 hrs (83.62 lux). The highest number of males was approached to virgin female at 18:50 hrs (15.94 lux) and terminated at19:20 hrs (0.26 lux).

Table 1. Calling females and male attraction by virgin females of *L. lepidophora* in the arecanut ecosystem in Gulukoppa, Hosanagara.

Time of day (hrs)	Number of males attracted by virgin female*	Calling female (%)*	Light intensity (Lux)
	Mean±SD	Mean±SD	Mean±SD
18:30-18:40	0.00	0.00	222.40±90.25
18:40-18:50	10.40±4.77	41.33±19.67	83.62±41.63
18:50-19:00	45.80±23.89	52.00±7.30	15.94±5.18
19:00-19:10	12.40±3.65	53.33±12.47	2.96±0.85
19:10-19:20	3.20±2.59	17.33±13.82	0.54±0.18
19:20-19:30	0.00	0.00	0.26±0.21
*N=75			

Mating behaviour of adult *L. lepidophora*

The sequential mating behaviour of adult *L. lepidophora* represented in figure 2. Before mating, males were emerged from the soil after dusk at 18:30 hrs onwards. Males made a repeated zigzag flight search for mates, by making buzzing sound throughout the garden at the height of approximately 15 to 20 feet. Copulating pairs (N=15) were observed with the aid of a head torch, in the areca nut field. At the same time, females emerged from the soil and flew for a short distance and settled on the host plants and took calling posture. Female beetles commenced abdominal expansion and contraction which may be an indication of starting secretion of semiochemicals. Subsequently, males were observed to fly toward calling females. The males were found to alight near the females with buzzing sounds. All females were observed to be receptive, and no case of mate refusal was observed (N = 15). The precopulatory phase began when the male approached the female. The male then moved closer to the female and immediately mounted. Female retracted her elongated abdomen to stop calling when male beetle mounted her. The male moves around the female and mounts her by climbing on to her abdomen. Male completely moved on her and held the thorax region with forelegs. Next, the male moved in a posterior direction as the abdomen bent ventrally and the aedeagus was extruded and lifted the female

abdomen by its hind legs. In the copulatory phase, the male inserted its genitalia into the female abdomen. When the genitalia had successfully joined, the male raised all of its legs, turned its body ventrally and became suspended by the genitalia. The copulatory phase ended with the withdrawal of the aedeagus from the genital chamber of the female. In the postcopulatory phase, the male dismounted immediately after genital retraction. Soon after copulation, males returned to the soil by dropping, while females stayed for a few minutes on the surface and flew to the soil for oviposition. Most unmated males also returned to the soil shortly after the active flight period at dusk. The copulation of fifteen beetles was observed between 18:47 hours to 19:05 hrs. The first termination of copulation occurred at 19:55 hrs and the last at 20:29 hrs. Copulation lasted for 74.60±6.61 min on average (mean±SD; range 64-85, N = 15). Copulatory phase was larger than the precopulatory 2.73±1.58 min on average (mean±SD; range 1-6, N = 15) and postcopulatory phase 1.67±0.90 min on average (mean±SD; range 1-3, N = 15) (Table 2).

Table 2. Mating duration of female beetles in the field during-2016.

Parameters	Minutes	
	Mean±SD#	Range
Pre copulation	2.73±1.58	1 -6
Copulation	74.60±6.61	64-85
Post copulation	1.67±0.90	1-3
#N=15		

Egg laying pattern of field-collected mated females of *L. lepidophora*

In a pooled analysis of 2015 and 2016, the longevity of female ranged from 11-28 days with an average of 18.52±4.91 days. The pre-oviposition period, Oviposition period and postoviposition period of an adult female with an average of 9.56±2.04 days (Mean±SD, range 4-13 days), 3.72±2.35 days (Mean±SD, range 1-11 days) and 5.24±4.67 days (Mean±SD, range 1-13 days), respectively (Table 3).

Table 3. Fecundity and longevity of *L. lepidophora* females during 2015-16 and 2016-17.

Parameter	2015*		2016**		Pooled#	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Pre-oviposition period (Days)	4-11	9.31±2.02	8-13	9.83±2.12	4-13	9.56±2.04
Oviposition period (Days)	1-11	3.92±2.72	1-7	3.50±1.98	1-11	3.72±2.35
Post-oviposition period (Days)	1-13	7.31±4.80	1-13	3.00±3.44	1-13	5.24±4.67
Fecundity (Eggs/female)	1-32	15.69±10.12	4-31	19±8.23	1-32	17.04±9.37
Adult longevity (Days)	11-28	20.54±5.13	11-24	16.33±3.73	11-28	18.52±4.91
*N=13; **N=12; #N=25						

Clutch size in relation to the clutch number

Data from egg laying pattern on a number of eggs in first, second, third, fourth and fifth clutches produced by females are summarized in Table 4. Mean number of eggs per clutches decreased from first through second, third fourth to the fifth (Mean±SD: first clutch, 8.36±6.18; range 1-20, Second clutch, 5.52±3.20, range 1-13, Third clutch 5.47±3.27, range 1-14, fourth clutch 2.29±1.80, range 1 to 6 and fifth clutch 3.20±1.60, range 1-6).

Table 4. Clutch size and mean number of eggs laid by female beetle *L. lepidophora* in laboratory condition.

Clutches	Mean number of eggs/ clutches	
	Mean±SD	Range
1	8.36±6.18	1 -20
2	5.52±3.20	1 - 13
3	5.47±3.27	1-14
4	2.29±1.80	1 - 6
5	3.20±2.17	1-6
*N=25		

Inter- clutch interval in relation to the clutch number

The number of days between successive clutches changed over time (Mean±SD). Time to between first and second clutch with an average of 41.05±27.66 hrs (N=21), between second and third clutch with an average of 37.09±43.41 hrs (N=11), between third to fourth clutch with an average of 37.71±18.88 hrs (N=7) and between fourth to fifth clutch with an average of 32±18.88 hrs (N=3) (Table 5).

Table 5. Inter clutch interval in relation to a clutch number of adult female beetle *L. lepidophora* in laboratory condition.

Clutches	Inter clutch interval (Hours)
	Mean±SD
1-2*	41.05±27.66
2-3**	37.09±43.41
3-4#	37.71±18.88
4-5##	32±18.88
*N=21, **N=11, #N=7, ##N=3	

DISCUSSION

Females are more active at around 18:40-19:20 hrs, where light intensity fell to 83.62 lux (Fig. 1). Present findings are in confirmation with Arakaki et al, (2004b) where *Dasylepida ishigakiensis* females were observed to emerging from the soil at 18:30-19:00 hrs and light intensity was less than 300 lux. Peak emergence of adult beetles occurred between 19:00 and 20:00 hrs. The emergence of *L. lepidophora* began at ~16:30 when illuminance fell below 65.5±12.5 lx and extended till 2100 (2.5±0.5 lx) (Kalleshwaraswamy et al, 2016). Further studies needed to confirm the relationship between the emergence of adult beetles and light intensity.

In this study, emerged females of *L. lepidophora* showed an abdominal expansion and contraction, indicating a typical sequence of calling behaviour to males and secrete sex pheromone. Similar behaviour was observed in *D. ishigakiensis* (Arakaki et al, 2004b) and *Lepidiota mansueta* (Bhattacharyya et al, 2015). Female *Exomala orientalis* (Waterhouse), also exhibited leg raising and stroking, which may aid in the dissemination of the pheromone (Facundo, Linn, Villani, & Roelofs, 1999). Precopulatory, copulatory and postcopulatory mating behaviour of *L. lepidophora* was closely matched with the *Dasylepida ishigakiensis* (Arakaki et al, 2004b; Fukaya et al, 2009; Harano et al, 2010; Tokuda et al, 2010) and *Lepidiota mansueta* (Bhattacharyya et al, 2015). Male and female of *Liogenys fusca* emerged between 19:00 and 23:30 h

and copulation occurred between 19:30 and 21:00 hr, and characterized by a typical behavioural sequence (Rodrigues, Moron, Gomes, & Bento, 2016). Whereas, soon after the emergence, both sexes leading to rapid mounting and successful mating on host plants. Copulation lasted for about 75 min. This duration was longer than those for other scarab species: 20 min in the green chafer, *Anomala albopilosa sakishimana* (Arakaki et al, 2004a) and the oriental beetle, *Exomala orientalis* (Waterhouse) (Facundo et al, 1999). It was less than those of scarab beetles like *Dasylepida ishigakiensis* (2 hours) (Arakaki et al, 2004b) and 85 min in the *Phyllophaga cuyabana* (Moser) (Oliveira & Garcia, 2003), *Lepidiota mansueta* (Bhattacharyya et al, 2015) and 15 min and 8 min in case of Chinese rose beetle *Adoretus sinicus* Burmeister (Hession, Arita, Furutani, & Fukada, 1994) and *Liogenys fusca* (Rodrigues et al, 2016), respectively. The male and female beetles of *L. lepidophora* exhibited protandrous type (males emerging before females) of emergence pattern during the emergence period (Kalleshwaraswamy et al, 2016)**.** Soon after the emergence of female, the large number of males were approached and leads to rapid mounting and mating on nearby plants or soil surface. This study indicated males and females of *L. lepidophora* emerged from soil specifically for mating. Hence, pheromone may be useful for management of areca nut white grub. This will be an aid to develop synthetic sex pheromone for mating disruption and mass trapping of male beetles.

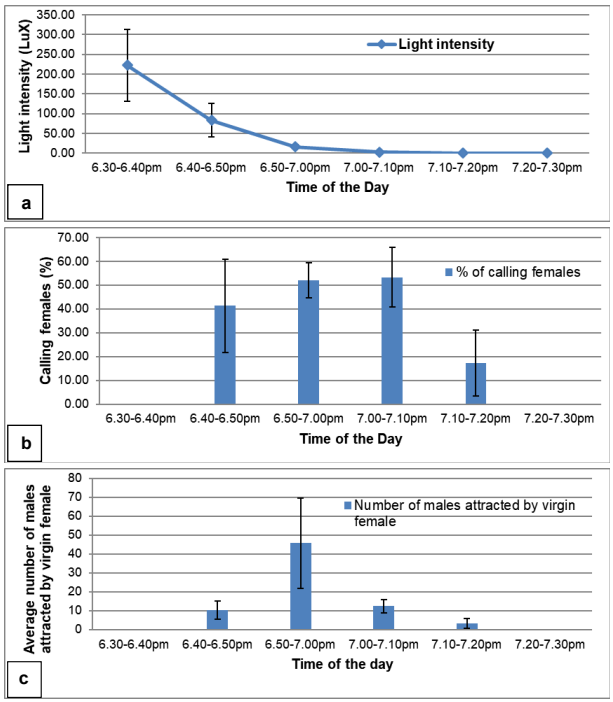

Figure 1. Calling female and male attraction by virgin females of *L. lepidophora* in the areca nut ecosystem in Gulukoppa, Hosanagara, a) light intensity, b) percentage of calling female (N=75), c) number of males attracted by virgin females.

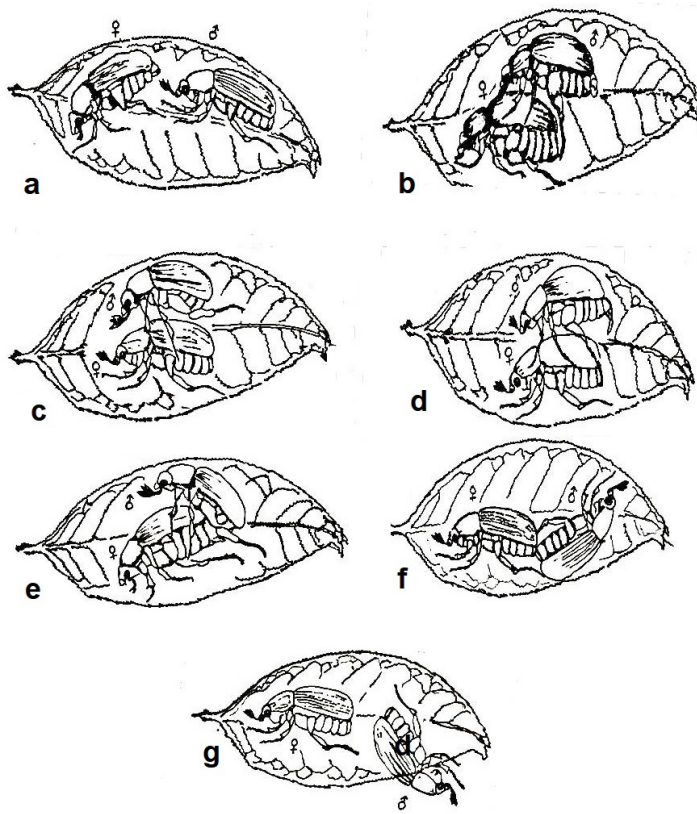

Figure 2. Mating behaviour represented in sequence of *L. lepidophora*. a) male approaches female, b) male mounts the female, c) male moves anteriorly on her and holds the thorax region with forelegs, d) male moved in a posterior direction as the abdomen bent ventrally and the aedeagus was extruded,e) male probes the end of the female's abdomen with his everted aedeagus, f) copulation-male turned its body ventrally and became suspended by the genitalia, g) dismounting and separation.

Experiment on egg laying pattern indicated that female waited on an average 9.56±2.04 days before starting of oviposition, after which time they began to lay the varying number of eggs at an average clutch interval of approximately 24-48 hours. Females of Japanese beetle *Popillia japonica* did not initiate oviposition until several days after emergence, and the number of eggs laid remained relatively constant over time (Timmerman, Switzer, & Kruse, 2000). Female areca nut white grub *L. lepidophora* lay on an average 17.04±9.37 eggs per female (range 1-32) over the one-month adult lifespan. Female laid eggs in multiple clutches after mating with one male. In our study, single mating is enough to fertilize all the eggs. The eggs laid by the female decreased in successive clutches (Fig. 3) may reflect either a cost of present reproduction on future fecundity (Bell & Kofoupanou, 1985), a reduction in overall performance and fecundity with increasing age of female (Park, Gustafsson, & Moreno, 1992). Fecundity of longhorn beetle *Cerambyx welensii* was decreasing

over time and showed a fluctuating synovigenic pattern (Torres-Vila, Mendiola-Diaz, Conejo-Rodriguez, & Sanchez-Gonzalez, 2016). Many protandrous species lay only one clutch of eggs (Wiklund & Forsberg, 1991) and those species that lay more clutches usually only have two to three clutches with the majority of eggs being laid in the first clutch (Milne, 1960). Decisions on clutch size are amenable to the programming approach because after each oviposition the reproductive state of the female has changed and hence if eggs or reserves are limiting, the optimal size of the next clutch may also have changed (Mangel, 1987). Several internal factors are responsible for clutch size in female beetles like rates of egg maturation, number of mature eggs, number of immature eggs, a capacity of oviducts, energy reserve and also the fitness of females (Wilson, 1989; Griffiths, 1990). Clutch size increases the reproductive success of the female. The present studies were helpful in the planning of integrated pest management strategies.

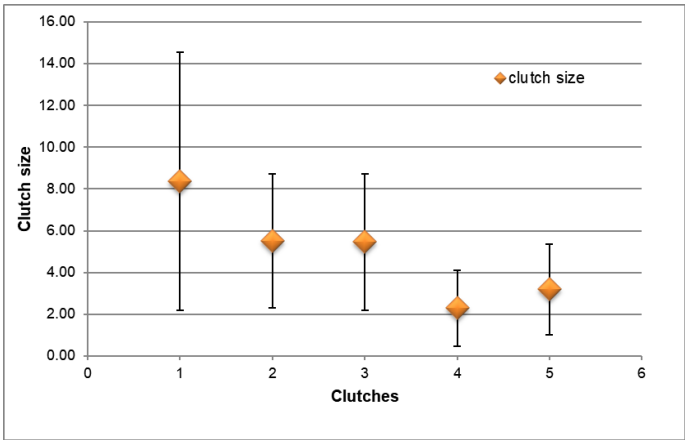

Figure 3. Number of eggs in the different clutches as an egg laying pattern.

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Effects of Host Plant Cultivar and Insecticide Type on Rice Damage and Growth of *Chilo suppressalis* (Lepidoptera: Crambidae) Larvae in Semi-field Conditions

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ABSTRACT

Rice striped stem borer, *Chilo suppressalis* (Walker, 1863), is considered as a major destructive pest in paddy fields in the most regions of Iran. In this study, the impact of the rice cultivars, Tarom and Shiroudi, and the insecticides, cypermethrin, tebufenozide and the emulsion and granule formulations of diazinon were examined on the damage indices, percentages of dead heart and whitehead. The study was performed as factorial randomized complete block design using artificial infestation in semi-field conditions in Amol County. No significant interaction was found between rice cultivar and insecticide for all indices in both vegetative and reproductive stages ($P>0.05$). The results of main effects in both phenological stages indicated that the weight of live larvae and damage indices were significantly higher on Tarom than those of Shiroudi ($P\leq0.05$), while no significant difference was detected in larval mortality on the cultivars ($P>0.05$). The main effects of insecticide were significant for all biological and damage indices ($P\leq0.05$). The efficacies of tebufenozide and cypermethrin in terms of larval mortality were 47.57 and 74.90 % at vegetative stage reaching to 79.15 and 92.08% at reproductive stage, respectively. The

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lowest efficacy in terms of larval mortality (73.33%) was obtained on plants treated by diazinon emulsion. At reproductive, the survived larvae weighed 170.80 mg.seedling^{-1} and the corrected whitehead index compared to the control reached 50.04% on plants treated by diazinon emulsion. According to the results, tebufenozide, regarding its relatively high efficacy and environmental compatibility, is suggested to be assessed further for integrated *C. suppressalis* management in the field conditions.

Keywords: Striped stem borer, Dead heart, Whitehead, Rice cultivar, Larval weight, Insecticide efficacy

INTRODUCTION

Rice (*Oryza sativa* L.) is considered as a primary food source in Iran and worldwide. (Tabari, Fathi, Nouri-Ganbalani, Moumeni, & Razmjou, 2017). Mazandaran province located in the north of Iran is the largest rice-producing area of Iran, with 235,000 hectares of rice cultivation (Youseftabar, Heidari Sharifabad, & Majidi Heravan, 2020). The striped stem borer*, Chilo suppressalis* (Walker, 1863), is one of the main destructive pests of the rice crop in Iran (Tabari et al., 2017) and the world (Lei, Zhang, Yun, Zhou, & Peng, 2020). This insect was introduced as a key pest in rice field of the Northern Iran in 1973 (Amooghli-Tabari et al., 2015). The adult insects lay their eggs on blade and sheath of the rice leaves in the nursery and field. The larvae enter into leaf sheath and feed on tissues causing yellowing and withering of tillers or dead heart at vegetative stage, usually 30 to 45 days following rice transplanting. The larvae also conduce to whitehead later in the reproductive stage of rice crop (Amooghli-Tabari et al., 2015). If *C. suppressalis* is not controlled in accurate time, the yield will be considerably reduced. Annual pest damage has been reported variably from 5 to 60% (Tabari et al., 2017).

The simultaneous using pest-resistant cultivars and insecticides has been suggested as IPM strategy in many injurious pests (EL-Gammal, EL-khyat, Abd El-Zaher, & Morsy, 2022). The use of resistant cultivars in pest control is perceived as a friendly environmental method (Tabari et al., 2017). In the present study, the commercial Iranian rice cultivars, Tarom and Shiroudi were used, previously reported as susceptible (Amouoghli Tabari et al., 2005) and tolerant (Mohadesi et al., 2009) to *C. suppressalis,* respectively. From the agronomic point of view, these cultivars are respectively characterized as early and medium maturity cultivars (Oskou, Nasiri, Omrani, & Zare, 2016). Through classical plant breeding method by crossing two rice cultivars (Tarom Dilmani × Khazar), the cultivar Shiroudi was introduced by the Iranian Rice Research Institute, known as a resistant to blast disease with high performance (Mohadesi et al., 2009). Tarom, a susceptible cultivar to *C. suppressalis*, has a lower performance than Shiroudi and known as a native and odorous rice, widely cultivated in the north of Iran (Tabari et al., 2017).

The use of chemical insecticides has been the main *C. suppressalis* controlling strategy in Iran and majority parts of the world so far (Mirhaghparast, Zibaee, Sendi, Hoda, & Fazeli-Dinan, 2015). Among the examined insecticides in present study, cypermethrin has not yet been registered for controlling of rice pests, although its use is common in paddy fields (Singh & Singh, 2020). Cypermethrin, as a synthetic pyrethroid insecticide, is a long-lasting and environmentally hazardous insecticide with

residual effect on rice husk (Teló et al., 2017). Diazinon, as a common organophosphate insecticide, has a long history of use in the paddy fields of northern Iran to control *C. suppressalis* (Mirhaghparast et al., 2015). In Mazandaran province, it widely enters water and environment and causes contamination in food chain (Won & Yang, 2015). In recent years, tebufenozide has been introduced as a specific and ecdysone agonist insecticide against lepidopteran pests including *C. suppressalis* (Roscoe, Forbes, Lamb, & Silk, 2020). Tebufenozide interferes with the hormonal control and molting of lepidopteran larvae, while it has low toxicity to non-target organisms (Roscoe et al., 2020).

In the current circumstance that increasing of crop production through expanding the cultivated area of crops, especially rice, has faced with limitation of climatic, social and financial resources in Iran, it is necessary to improve an integrated rice striped stem borer management relying on available and common resources to reduce pest damage. The aim of present research was to evaluate the efficacy of four common insecticides in controlling of the striped stem borer on Tarom and Shiroudi cultivars in Amol paddy fields using examination of biological and damage indices.

MATERIAL AND METHODS

The current research was conducted in the Rice Research Institute of Iran, Deputy of Mazandaran, Department of Plant protection, located in Amol county (longitude: 52° 23' E latitude: 36° 28' N and height: 23.7 meters above sea level) in 2019, based on the factorial randomized complete block design. The first factor consisted of Tarom and Shiroudi rice cultivars and the second one included the insecticides cypermethrin (EC 40%, Golsam company of Gorgan[1]), Tebufenozide (SC 20%, Nippon Soda Company of Japan[2]), Diazinon emulsion (EC 60%, Golsam company of Gorgan), Diazinon granule (G 10%, Golsam company of Gorgan) and the control (water only). The experiments were performed in semi-field conditions inside the screen-confined pots in 5 replicates.

Culturing of the host-plants

The seeds of Tarom and Shiroudi were obtained from Iran Rice Research Institute, Deputy of Mazandaran and were used in transplanting nursery for planting. 15% salt water solution was used to remove unhealthy rice seeds. After thoroughly rinsing with water, the seeds were soaked in water for 24 h. Disinfection of seeds was performed using carboxin thiram (40% FS) at 3 ml.kg^{-1}seed for 24 h. The seeds of Shiroudi and Tarom were kept in a hothouse at 35±1°C for 3 to 5 days, respectively. The germinated seeds were transferred to the nursery to prepare transplants. Transplants were planted in plastic pots with 30 cm diameter and 10 L volume (Tabari et al., 2017). Three seedlings with 3 to 4-leaves (25 to 30-day old transplants) in a pot were regarded as an experimental unit. After drying and crushing, the soil originally collected from the paddy fields of surrounding area was used to fill the pots up to a 5 cm from their surfaces. Agronomic cares were carried out based on the technical recommendation

[1]Golsam Gorgan Chemical Office Address: 20th Jorjan alley Jorjan Blvd, Gorgan, Iran.
[2]Imported by Raha Andish Kavan Chemical Office Address: No. 18, North Khazar St. Elahiyeh, Tehran, Iran.

of the Iranian Rice Research Institute. Irrigation was done every other day until the middle of plant ripening. Weeding was done manually at intervals of every 15 days. Fertilization was carried out using urea (46% N_2), triple superphosphate (46% P_2O_5) and potassium sulfate (K_2O 50%) at the rate of respectively 3, 3 and 4 $g.pot^{-1}$ mixed with soil before transplanting. Furthermore, 25 days after transplanting, top-dressing was done with 3 $g.pot^{-1}$ of urea and 40 days later with 3 $g.pot^{-1}$ of each of urea and potassium sulfate fertilizes. (Majidi-Shilsar, 2015). The experimental pots were covered by net and kept outside in field conditions with a distance of 100 cm until the end of experiments.

Rearing of rice striped stem borer larvae

Male and female insects were collected from Amol paddy fields using light traps and settled on Fajr rice cultivar .The use of this cultivar in insect culture was due to prevention of the rice striped stem borer adaptation to the experimental cultivars of Tarem and Shiroudi. The egg masses were obtained in laboratory as described by Majidi-Shilsar (2015). Newly-hatched larvae were used for infestation of the experimental rice seedlings.

Experiments Implementation

Two weeks after transplanting, 15 newly-hatched larvae per rice seedling (totally 45 larvae per pot) were transferred on the leaf auricle so that they had access willingly to the feeding area. To ensure the larvae settlement and escaping prevention, a cylindrical mesh cage (30 × 90 cm) was laid on each seedling for 24 h (Tabari et al., 2017). One week after artificial infestation, i.e. 21-day post-transplanting, insecticidal treatments were performed using a 2 L handheld sprayer. The insecticides were applied according to the manufacturer recommendations as 0.175 $L.ha^{-1}$ of cypermethrin, 0.75 $L.ha^{-1}$ of tebufenozide, 2 $L.ha^{-1}$ of diazinon emulsion and 15 $kg.ha^{-1}$ of diazinon granule. The diazinon granule was applied as mixed with dry sand into water of pots for optimal coverage as well as better absorption by the roots.

Measurement of Insecticidal efficacy and damage indices

In the vegetative stage of rice plant, 21 days after artificial infestation of host plants with larvae, one of the three seedlings of experimental pots was excised at the soil level. The number of tillers with dead heart symptoms (Dh) was counted and recorded as percentage using the equation, Dh%=(No. of Dh/total No. of tillers) ×100 (Mahapatra & Nanda, 1996). The number and weight of live larvae were also recorded. Moreover, the percentage of larval mortality in each seedling was recorded compared to the initially artificial infestation.

In reproductive stage of rice plant, following excision of the second seedling, the number of tillers with whitehead symptoms (Wh) was counted and recorded as percentage using the equation Wh%=(No. of Wh/total No. of tillers) ×100. The number and weight of live larvae and the mortality percentage compared to the initial plant

infestation with larvae were recorded. At this phenological stage, the number of pupae on the seedling was also counted.

The efficacy of insecticide treatments was assessed using Abbott's formula, by which the percentage of the damage indices and larval mortality were corrected compared to the counterpart data in control (Abbott, 1925).

Data analysis

Prior to the statistical analysis, the normality and homogeneity of variances were subjected to Jarque-Bera Test (Jarque & Bera, 1987) and Levene's Test (Levene, 1960), respectively, and if necessary, the data were converted to $(x+1)^n$. Analyses of variance (ANOVA) were performed on all biological and damage indices in factorial randomized complete block design. Comparisons of means were made using Fisher's protected least significant difference (LSD) procedure. Pearson correlation analysis was performed between indices of damage and biological characteristics. All statistical analyses were performed using SAS software version 9.4 (SAS Institute, Inc, 2004).

RESULTS

Assessment of plant damage and biological characteristics in the vegetative stage of rice

The results of ANOVA in the vegetative stage of the rice plant demonstrated that the main effects of cultivar and insecticide on dead heart ($F=25.26$; $df=13,36$; $P\leq0.0001$), the number of live larvae ($F=45.1$; $df=13,36$; $P\leq0.0001$), live larvae weight ($F=47.23$; $df=13,36$; $P\leq0.0001$) and larval mortality ($F=39.33$; $df=13,36$; $P\leq0.0001$) were significant. The block effect and the interaction effect of cultivar × insecticide on all variables were not significant ($P>0.05$). The results of main effects of cultivar revealed that the dead heart percentage, the number of survived larvae and the larval weight were significantly higher in Tarom than those in Shiroudi (Table 1).

Table 1. The main effect of cultivar on the biological characteristics of *C. suppressalis* larvae and their damage indices (Mean±SE) [β] in the vegetative stage of rice plant [€].

Rice cultivar [¥]	Dead heart (%)	Live larvae gain (per seedling)		Mortality (%)
		Number	Mass (mg)	
Shiroudi	10.67±1.08 b	7.48±0.73 b	144.32±14.35 b	50.13±4.87 a
Tarom	23.76±2.23 a	8±0.74 a	203.44±19.23 a	46.67±4.94 a

[SE] Standard Error
[β] Within each rice cultivar, all insecticide-treated data (Including control) were pooled (n=25).
[€] Sampling made at 14-day post-insecticide application (35 day post-transplanting).
[¥] Each 14-day old seedling (3 seedings per pot) was infested by 15 newly-hatched larvae.
Means followed by the same letters in each column are not significantly different (LSD Test, $P<0.05$).

The main effects of insecticide indicated that the control- and cypermethrin- treated plants had respectively the highest (30.74%) and lowest (7.52%) percentages of dead heart. The lowest number (3.6) and weight of survived larvae (80.2mg) on plants were achieved through the cypermethrin application. The highest percentage of larval mortality was associated with cypermethrin treatment (76%) (Table 2).

Table 2. The main effect of insecticide on the biological characteristics of *C. suppressalis* larvae and their damage indices (Mean±SE) [β] in the vegetative stage of rice plant [€].

Treatment [¥]	Dead heart (%)	Live larvae gain (per seedling)		Mortality (%)
		Number	Mass (mg)	
Tebufenozide (Sc)	16.64±2.62 b	7.5±0.22 b	168.7±10.83 b	50±1.49 c
Diazinon (Ec)	15.03±2.32 b	6.4±0.31 c	142.2±10.18 c	57.33±2.04 b
Diazinon (G)	16.14±2.44 b	6.9±0.28 bc	154.3±10.57 bc	54±1.85 bc
Cypermethrin (Ec)	7.52±1.15 c	3.6±0.22 d	80.2±7.52 d	76±1.47 a
Control	30.74±3.72 a	14.3±0.15 a	324±17.3 a	4.67±1.02 d

[SE] Standard Error
[β] Within each insecticide-treated experimental unit, all rice cultivars data were pooled (n=10).
[€] Sampling made at 14-day post-insecticide application (35 day post-transplanting).
¥ Each 14-day old seedling (3 seedings per pot) was infested by 15 newly-hatched larvae.
Means followed by the same letters in each column are not significantly different (LSD Test, P<0.05).

Cypermethrin possessed the highest percentage of efficacy in decreasing the dead heart percentage and the survived larvae weight on rice plants, followed by diazinon emulsion. Tebufenozide represented the least insecticidal efficacy (Fig. 1).

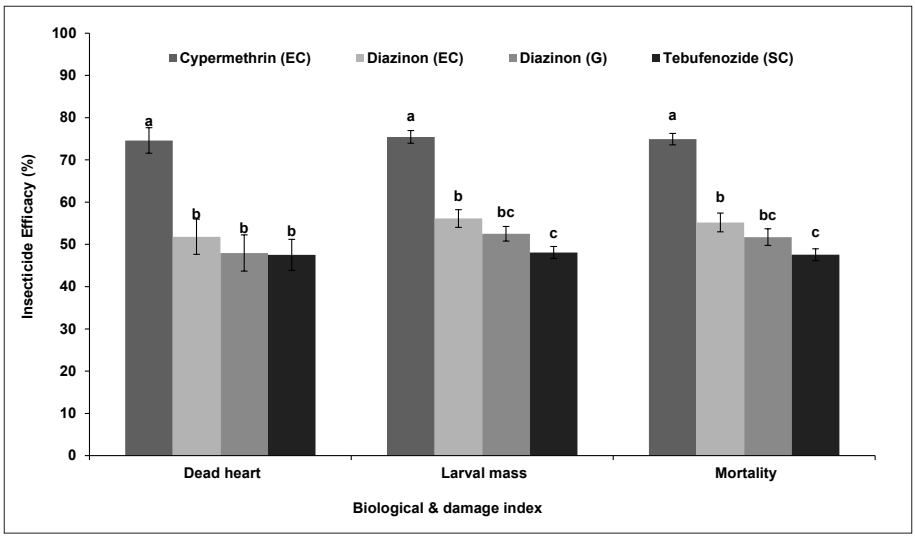

Figure 1. The percentage of insecticide efficacy (mean ± SE) at vegetative stage of the rice plant in terms of damage indices of *Chilo suppressalis* larvae. Within each insecticide-treated experimental unit, all rice cultivar data were pooled (n=10). The insecticide efficacy was calculated as corrected-damage in control using Abbott's formula (1925). (Within each damage index, columns carrying different letters are significantly different (P≤0.05, LSD Test).

Assessment of plant damage and biological characteristics in the reproductive stage of rice

The results of ANOVA in the reproductive stage of the rice plant showed that the main effects of cultivar and insecticide on whitehead (F=67.99; df=13,36; P≤0.0001), the number of live larvae (F=56.06 ; df=13,36; P≤0.0001), weight of live larvae (mg) (F=32.62; df=13,36; P≤0.0001), larvae mortality (F=42.64; df=13,36; P≤0.0001) and

the number of pupae (F=42.13; df=13,36; P≤0.0001) on the rice plant were significant. The effects of block and cultivar × insecticide interaction were not significant on the all studied features (P>0.05). The results of main effect of cultivar revealed that the whitehead percentage and the larval weight were significantly higher in Tarom than those in Shiroudi (Table 3).

Table 3. The main effect of cultivar on the biological characteristics of *C. suppressalis* larvae and their damage indices (Mean±SE) β in the reproductive stage of rice plant €.

Rice cultivar ¥	Whitehead (%)	Live larvae gain (per seedling)		Mortality (%)	Pupa (No. seedlings-1)
		Number	Mass (mg)		
Shiroudi	17.73±1.86 b	4.84±0.92 a	195.84±37.32 b	67.73±6.13 a	4.12±0.96 a
Tarom	44.47±5.09 a	5.24±0.94 a	260.12±47.08 a	65.07±6.26 a	4.6±1.01 a

SE Standard Error
β Within each rice cultivar, all insecticide-treated data (Including control) were pooled (n=25).
€ Sampling made at 69 and 44 days post-insecticide application in Shiroudi and Tarom, respectively.
¥ Each 14-day old seedling (3 seedinligs per pot) was infested by 15 newly-hatched larvae.
Means followed by the same letters in each column are not significantly different (LSD Test, P<0.05).

The main effects of insecticide indicated that the control- and cypermethrin- treated plants had respectively the highest (62.16%) and lowest (13.77%) percentages of whitehead. At the end of the experiment, the lowest number (1.1) of survived larvae and their weight (54.1mg) were detected on the plants sprayed with cypermethrin. However, the number of pupae on the tebufenozide-sprayed plants was not significantly different from that on cypermethrin-sprayed plants. (Table 4).

Table 4. The main effects of insecticide on the biological characteristics of *C. suppressalis* larvae and their damage indices (Mean±SE) β in the reproductive stage of rice plant €.

Treatments ¥	Whitehead (%)	Live larvae gain (per seedling)		Mortality (%)	Pupa (No. seedlings-1)
		Number	Mass (mg)		
Tebufenozide (Sc)	20.88±3.23 c	2.9±0.18 c	119±9 c	80.67±1.2 b	0.8±0.2 c
Diazinon (Ec)	29.67±4.08 b	3.7±0.15 b	170.8±10.49 b	75.33±1.02 c	3.5±0.22 b
Diazinon (G)	29.01±4.64 b	3.6±0.16 b	165.8±11.66 b	76±1.09 c	3.1±0.18 b
Cypermethrin (Ec)	13.77±1.93 d	1.1±0.18 d	54.1±9.76 d	92.67±1.2 a	0.7±0.15 c
Control	62.16±9.48 a	13.9±0.18 a	630.2±27.62 a	7.33±1.2 d	13.7±0.21 a

SE Standard Error
β Within each insecticide-treated, all rice cultivars data were pooled (n=10).
€ Sampling made at 69 and 44 days post-insecticide application in Shiroudi and tarom, respectively.
¥ Each 14-day old seedling (3 seedings per pot) was infested by 15 newly-hatched larvae.
Means followed by the same letters in each column are not significantly different (LSD Test, P<0.05).

Cypermethrin was represented by the highest efficacy in terms of decreasing the whitehead percentage and the survived larvae weight on the rice plant, followed by tebufenozide treatment (Fig. 2).

The positive and significant correlations were detected between dead heart percentage and larval weight in the vegetative (r=0.917) and reproductive (r=0.766) stages of rice plants (P≤0.0001). Furthermore, there were negative and significant correlations between whitehead percentage and larval mortality in the vegetative (r=-0.699) and reproductive (r=-0.771) stages of rice plants (P≤0.0001).

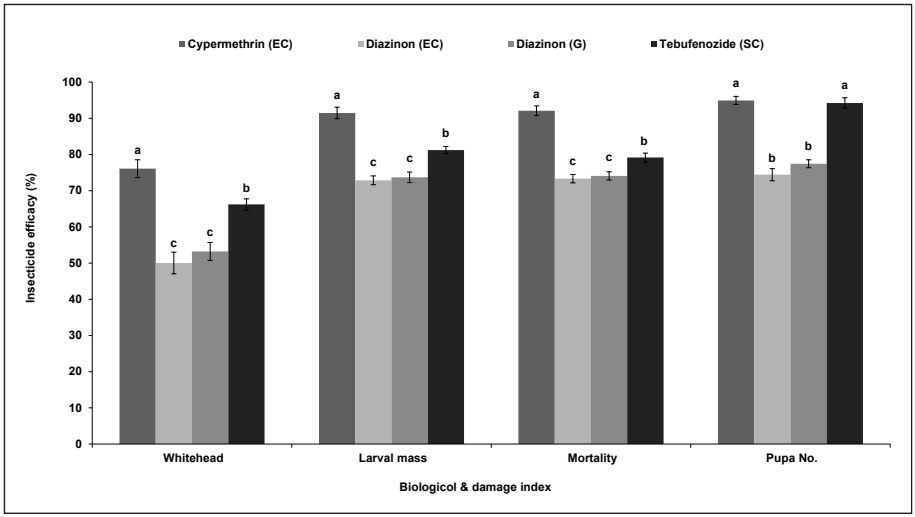

Figure 2. The percentage of Insecticide efficacy (mean ± SE) at reproductive stage of the rice plant in terms of damage indices of *Chilo suppressalis* larvae. Within each insecticide-treated experimental unit, all rice cultivar data were pooled (n=10). The insecticide efficacy was calculated as corrected-damage in control using Abbott's formula (1925) (Within each damage index, columns carrying different letters are significantly different (P ≤ 0.05, LSD Test).

DISCUSSION AND CONCLUSION

The current study represented that the cultivar × insecticide interaction effect on *C. suppressalis* larvae was not significant in all of the examined features (P>0.05). Wilson et al. (2020) in their research on the interaction effects of cultivar, nitrogen fertilization and insecticide on sorghum field infested with the sugarcane aphid, *Melanaphis sacchari* (Zehntner, 1897), showed that the interaction effect of sorghum cultivars on the aphid during the application of Flupyradifurone insecticide in South Carolina was not significant. In contrast, many researchers have demonstrated that the interaction effect of insecticides application and relative resistant plants on piercing-sucking insects such as aphids led to a decrease in the pesticides concentration for the pest controlling on resistant cultivars (Heinrichs, Fabellar, Basilio, Wen, & Medrano, 1984; Kea, Turnipseed, & Carner, 1978; Taksdal, 1992). Reduction in amount of insecticide application against the reared insects on the cultivars with relative resistance, ultimately elicits costs reduction (Heinrichs et al., 1984), a reduction in detrimental environmental effects on natural enemies (Van Emden, 1990) and predation reinforcement (Nicol, Wratten, Eaton, & Copaja, 1993), reducing insecticides resistance to pests (Mohamad & Van Emden, 1989) and a possibility of delaying resistance-breaking in pest-resistant cultivars (Van Emden, 1991). Contrary to the above-mentioned researches, no interaction effect was observed between insecticide and rice cultivar, presumably owing to low difference in resistance between rice varieties, particularly in the semi-field conditions of the present study.

The results revealed that at the end of the experiment the number of *C. suppressalis* pupae on the rice plants was higher on Tarom cultivar than Shiroudi, even though the difference was not statistically significant (Table 4). According to Tabari et al. (2017), Shiroudi with more tillers showed higher antibiosis resistance to *C. suppressalis* compared to Tarom cultivar. Shiroudi was recommended as a useful rice cultivar in integrated pest management (IPM) as well as breeding programs. Ghaninia and Amooghli-Tabari (2016) also demonstrated that the tendency of *C. suppressalis* adults toward Shiroudi is less than Tarom.

The main effects of insecticide in the rice reproductive stage represented cypermethrin with the highest mortality and lowest larval biomass on rice plants (Table 4) and accordingly the highest efficacy compared to other insecticides (Figure 2). Horgan, Peñalver-Cruz, & Almazan (2021) in greenhouse conditions showed that cypermethrin decreased oviposition of the brown plant hopper, *Nilaparvata lugens* (Stal) in resistant and susceptible rice cultivars, leading to high controlling effect on nymph population. Nevertheless, Horgan et al. (2021) pointed out the adverse effects of cypermethrin including phytotoxicity, reduction of rice yield and tillers (Horgan et al., 2021). Cypermethrin was also reported as a carcinogenic and hazardous substance for environment in some countries (Singh & Singh, 2020). Thus, despite the high efficacy of cypermethrin on larvae in the present study, considering the reports of its adverse effects on health and environment in the literature, the use of this insecticide is not advised in the paddy fields.

The efficacy of tebufenozide particularly in the rice reproductive stage was more than 66% in terms of biological and plant damage indices (Figure 2). Tebufenozide has less detrimental effects on environment, especially against honeybees (Abramson, Squire, Sheridan, & Mulder Jr, 2004). He et al. (2008) demonstrated that populations of the 4th-instar *C. suppressalis* larvae were susceptible to tebufenozide in four regions of China, but in some regions, partial resistance to diazinon was detected. Roscoe et al. (2020) stated that tebufenozide act mainly in the molting stage of lepidopteran larvae. Tebufenozide needs more time to be effective on insect larvae (He et al., 2008). Moreover, in the present study, the slow effectiveness of this insecticide was revealed by comparing the results of biological and damage indices between the phenological rice stages (Figures 1 and 2). This insecticide belongs to the benzoylurea group, recommended for controlling the rice stripe stem borer (He et al., 2008; Yu et al., 2016). From this group, hexaflumuron was also suggested as an alternative insecticide to diazinon in paddy fields (Mirhaghparast et al., 2015). Additionally, the environmental compatibility of Tebufenozide and its safety for non-target arthropods have been reported (Roscoe et al., 2020).

According to the studies of Mirhaghparast et al. (2015), the efficacy of diazinon emulsion gradually decreased during the long-term application, probably due to selection pressure in repeated use of organophosphorus pesticides in paddy fields. Emphasizing the lack of *C. suppressalis* larvae resistance to diazinon in their studies, Alhosseini et al. (1998) indicated that the efficacies of diazinon granule were 79.7 and 64.01%, in terms of dead heart and whitehead percentages, respectively

(Alhosseini, Heidari, & Mostofi-Pour, 1998). In the present study under semi-field conditions, the efficacies of 47.97 and 53.23% on *C. suppressalis* larvae were achieved for diazinon granule in terms of the respective criteria at 14-day post-infestation. Hassani, Hajiqanbar, Jalaeian, Moharramipour, & Moharramipour (2022) reported an effectiveness of 60.92% under natural field conditions for diazinon granule in terms of dead heart at 12 days after insecticide application. The efficacy of diazinon granule based on the reduction of whitehead percentage was more than its emulsion formulation (Figure 2). In contrast, under natural field conditions and rice-based double-cropping system, Amiri et al. (2017)reported less efficacy of diazinon granule based on the same criteria compared to its emulsion formulation. Comparing the efficacies of diazinon between phenological stages of rice plants (Tables 2 and 4), the results indicated that diazinon caused to increase total larval biomass on plants at reproductive stage. Nevertheless, due to environmental and health hazards of diazinon formulations as well as their role in insecticidal resistance development in insect pests (Mirhaghparast et al., 2015)., their application have recently been prohibited in Iran (Plant_Protection_Organization, 2021) and some other countries (Won & Yang, 2015).

In order to assess plant damage or plant tolerance to C. *suppressalis* larvae, percentage dead heart and/or whitehead indices, preferably the latter, have been widely proposed (Amooghli-Tabari et al., 2015). In the present study, based on the index of white head percentage, the relative resistance of the Shiroudi cultivar to C. *suppressalis* larvae compared to Tarom was revealed (Table 3), confirming the previous findings (Ghaninia et al., 2016; Tabari et al., 2017)

Significant relationships were demonstrated between biological characteristics of larvae and damage indices. Likewise, Amooghli-Tabari et al. (2015) reported a positive and significant correlation between the whitehead percentage and the larval density on the seedling (Amooghli-Tabari et al., 2015). Hosseini, Babaeian, Bagheri, Khademian, & Hasan (2011) reported a negative and significant correlation between the number of tillers and the whitehead percentage.

In conclusion, the effects of different insecticides on rice cultivars to control *C. suppressalis* larvae were evaluated. The tolerance of Shirodi cultivar to the damage of larvae was indicated to be greater compared to Tarom. The insecticide tebufenozide, regarding its relatively high efficacy and the previous reports of environmental compatibility, is suggested to be assessed further for integrated *C. suppressalis* management in the field conditions.

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Efficacy of Food Attractants and Attract-and-Kill System to Control *Dacus ciliatus* (Loew) (Tephritidae: Diptera): New Recorded Pest in Northern Tunisia

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ABSTRACT

Some morphological features of *Dacus ciliatus* (Loew) reported for the first time in Tunisia were studied herein. Also during this study, the efficacy of food attractants and attract-and-kill devices were tested against *D. ciliatus*. To do so, McPhail® traps baited with diammonium phosphate (DAP©) and protein hydrolysate (Ceratrap©), already known as food attractants for fruit flies, were compared in zucchini and cucumber crops. A novel attract and kill system (Ceranock© female and male) was also applied in the two cited crops. Moreover, our data highlighted the usefulness of DAP© as effective food attractant with a high number of trapped adults compared to Ceratrap© baited traps. Few adults were recorded by Ceratrap© only in Zucchini treated crop 1 (0.42±0.49 and 0.28±0.69 respectively for males and females) compared to the other surveyed crops. Attract and kill system achieved good control of the pest by decreasing the number of captured flies in treated crops compared to the untreated crops. The use of attract and kill system showed promising results and can successfully be used to manage the lesser pumpkin fly in Tunisian cucurbit crops.

Keywords: The lesser pumpkin fly, DAP©, Ceratrap©, Ceranock®, trap catches, cucurbits.

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INTRODUCTION

The Ethiopian fruit fly, *Dacus ciliatus* (Loew) (Diptera: Tephritidae), is a serious pest of cucurbits (e.g. cucumber, zucchini, and melon) (Alagarmalai et al, 2009; Rempoulakis, Nemny-Lavy, Castro, & Nestel, 2016) already reported in many countries of Africa, Asia and the Middle East (Arghand, 1979; EPPO/CABI, 2006; Fetoh, 2009; Kamali, Karimi, Hosseini, Campos-Herrera, & Duncan, 2013). Recently, *D. ciliatus* was reported in Turkey and Iraq (Al-Muffti & Al-Maronsy, 2018; Çalışkan Keçe, Özbek Çatal, & Ulusoy, 2019). Various control methods are used to manage cucurbit fruit flies worldwide (Qureshi & Hussain, 1992; Hussein, El-Wakeil, & El-Sebai, 2006; Alagarmalai et al, 2009; Kamali et al, 2013; Rempoulakis et al, 2016; Mohammadpour, Faghih, & Araghi, 2017; Shaked et al, 2017). Current approaches for pest management based on behaviour-modifying chemicals are successfully applied for management of fruit flies (El-Sayed et al, 2009; Piñero, Mau, McQuate, & Vargas, 2009; Navarro-Llopis, Primo, & Vacas, 2012; Bouagga et al, 2014; Hafsi, Abbes, Harbi, Duyck, & Chermiti, 2015b). Among these approaches, there is attract-and-kill method which is considered as an efficient method thanks to its beneficial effects towards human health and biodiversity (El-Sayed et al, 2009; Piñero et al, 2009; Bouagga et al, 2014). Different forms of attract-and-kill systems, which consist of killing the target fly by the chemical product once attracted by the semiochemical lure, have already been tested for their efficacy worldwide (Piñero et al, 2009; Ryckewaert, Deguine, Brévault, & Vayssières, 2010; Bouagga et al, 2014; Hafsi et al, 2015a). As example, bait station systems were found to be efficient for the control of some Tephritidae species including *Bactrocera dorsalis* (Hendel), *B. cucurbitae* (Coquillett), and *Ceratitis capitata* (Wiedemann) **(**Piñero et al, 2009; Navarro-Llopis et al, 2012; Bouagga et al, 2012; Hafsi et al, 2015a).

To our best knowledge, there is no available data confirming the occurrence of *D. ciliatus* in Tunisia. From this point of view, this work aimed firstly, to study some morphological aspects of *D. ciliatus*. Secondly, we tested the efficacy of food attractants and attract and kill systems for the fruit fly management in zucchini and cucumber crops.

MATERIALS AND METHODS

Traps

McPhail® traps, provided by (Russell IPM®), were used to monitor fruit flies herein. The survey was conducted from 01/06/2020 to 24/08/2020 in cucurbit crops located in Takelsa region (Northeast Tunisia). Three Zucchini (cvs. Nesta) and two Cucumber (cv. Thomson) crops were fixed for this study. Each crop had an area of one ha. Traps were baited with two food attractants. The first attractant was DAP© (diammonium phosphate), while the second was Ceratrap© (composed of protein hydrolysate). Contents of traps were renewed weekly and the number of captured adult flies were counted and removed. Adults were transferred to the laboratory of entomology and acarology at National Agronomic Institute of Tunisia (INAT) and examined using a stereo microscope.

Attract-and-kill systems

Ceranock® female (bait station) and male (gel) supplied by (Russell IPM®), an innovative "attract-and-kill" technology for Med-fly control**,** were tested for their efficacy, in Takelsa region, where *D. ciliatus* was found. Experiments were conducted from 15/07/2020 to 24/08/2020 in Zucchini and Cucumber crops already described. All crops were planted in June 01, 2020. Distance between plants and rows were 50 cm and 100 cm respectively for each crop. No chemical treatments were done during the experiment period. As recommended by the supplier, 100 Ceranock® female per ha were hanged at a height of 50 cm on 100 cucurbits plant distributed homogeneously within each treated crop. Females were attracted to an attractant and killed by an insecticide after contact. The used attractant were protein hydrolysate and plant extract (5 g/bait station), while the insecticide was cypermethrin (0.01 g/bait station). According to the supplier, the tested device consisting on three components (plastic hook, plastic case and a sponge impregnated with attractant and insecticide) should be renewed every 120 days under natural conditions. Ceranock® male is a specialized wax emulsion formulation commercialized as a tube containing trimedlure (35%) and abamectin (0.5%). This product, was applied as droplets of paste (2 gr) put in a small plastic device attached on each plant at a height of 0.5 m. The gel was applied only once on plants located at the border of each crop.

Two Zucchini and one Cucumber crops were treated while one Zucchini and one Cucumber crops were left as untreated controls. Both treated and untreated crops contained two McPhail traps baited with DAP© and Ceratrap© for *D. ciliatus* monitoring during the experiment.

Morphological identification of *D. ciliatus*

Fruit flies were collected from McPhail® liquid traps and examined using a stereo microscope (EPPO, 2018). Morphological identification was made based on published Keys for fruit flies (White & Elson-Harris, 1992; Drew & Romig, 2013).

Statistical analysis

Levene and Shapiro-wilk test were applied to check the homoscedasticity and normality of the obtained data. The effect of factors 'treatment', 'crop' and 'food attractant' on fruit fly catches was analysed using a Generalized Linear Model (GLM) followed by one-way ANOVA (Duncan test at $P < 0.05$). Statistical analysis was performed using IBM SPSS Statistics for Windows version 21.0 (IBM Corp, 2012).

RESULTS

Morphological identification of *D. ciliatus*

Taxonomy

Adults belonging to the Genus *Dacus* were identified based on published keys for Tephritidae species (White & Elson-Harris, 1992; Drew & Romig, 2013). *Dacus ciliatus*

adults have a pale orange-brown color. Femur of mid leg has brown color. Katatergite and not the anatergite is covered by yellow spot compared to *D. frontalis*. Wings have a brown color with a narrow costal band. The distance between eyes slightly exceed 0.5 mm for *D. ciliatus* males and females.

Effectiveness of attract-and-kill system

Figure 1 indicates that the number of trapped males and females in DAP© baited traps was significantly higher than it recorded in Ceratrap© baited traps for Zucchini and cucumber crops. No adults were captured by Ceratrap© baited traps except for Zucchini treated crop 1 (0.42±0.49 and 0.28±0.69 respectively for males and females) (Figure 1). The interaction between treatment, crop and food attractant significantly influenced the number of captured *D. ciliatus* flies (F=4.48; df=9; p<0.001 and F=2.74; df=9; p=0.009) for males and females respectively). For DAP© baited traps, statistical analysis showed that there is a significant difference between treated and untreated crops for the number of captured males and females ($F_{4, 34}$=4.85; p<0.001 and $F_{4, 34}$=3.24; p=0.02) respectively for males and females). However, for Ceratrap©, there is only significant difference for the number of captured males ($F_{4, 34}$=4.50; p<0.001) and not for females ($F_{4, 34}$=1.00; p=0.42). Furthermore, there is a significant difference between DAP© and Ceratrap © for the number of captured adults in Zucchini crops ($F_{1, 41}$=16.78; p<0.001 and $F_{1, 41}$=17.65; p=0.001 respectively for males and females) and the Cucumber crops ($F_{1, 27}$=19.89; p=0.001 and $F_{1, 27}$=15.13; p=0.001 respectively for males and females).

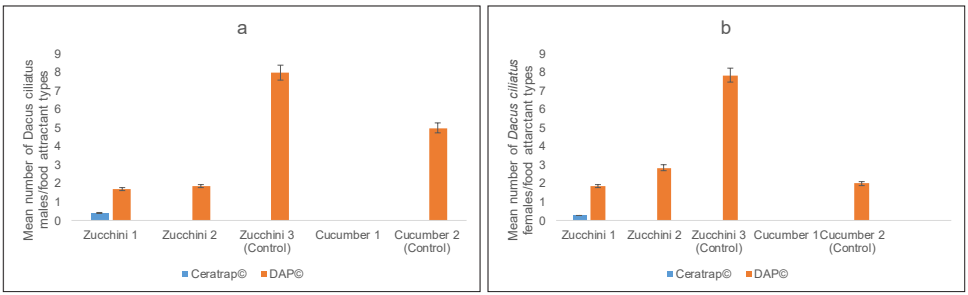

Figure 1. Comparison between two food attractants baited traps (DAP© and Ceratrap©) in treated and untreated crops. a) males, b) females.

DISCUSSION AND CONCLUSIONS

Fruit flies (Diptera: Tephritidae) are classified among the most destructive pests representing a limiting factor for safety food production in the world (Alagarmalai et al, 2009; Deploux & Dequine, 2015; Díaz-Fleischer, Pérez-Staples, Cabrera-Mireles, Montoya, & Liedo, 2017; Dias, Zotti, Montoya, Carvalho, & Nava, 2018). These pests can cause direct damage to fruits and vegetables by decreasing their quality values (Navarro-Llopis et al, 2012; Lasa & Cruz, 2014; Dias et al, 2018). Various factors (e.g. biology, available hosts…) can negatively influence the efficiency of control tactics

undertaken for flies' management (Dias et al, 2018). A correct identification of fruit flies using morphological or/and molecular methods, may represent a basic premise for an efficient and successful pest management strategy (Dias et al, 2018). Here, it has been shown that the distance between compound eyes slightly exceed 0.50 mm for *D. ciliatus* adults which confirm results of Fetoh (2009). Usually, pest prevention measures may represent a primordial step to be taken for a successful management of fruit flies (Aluja, 1999). Traps are used for fly monitoring which may help not only to estimate possible induced damages but also to judge the efficacy of control tools (Enkerlin, Lopez, & Celedonio, 1996; Eliopoulos, 2007). Despite the disadvantages of manufactured traps such as the high cost and time handling (Goldshtein et al, 2017), different trap types are commercialized to monitor fruit flies including *D. ciliatus*, worldwide (Mohammadpour et al, 2017; Shaked et al, 2017; Dias et al, 2018). As example, yellow electronic traps are proved to be attractive to *D. ciliatus* in melon plastic tunnels (Shaked et al, 2017). In the present study, we demonstrated that McPhail® traps filled with DAP© and Ceratrap© as food attractants can attract both *D. ciliatus* males and females. Also, DAP© are significantly more attractive to *D. ciliatus* compared to Ceratrap©. Despite the lack of information regarding the use of attractants and traps for *D. ciliatus* monitoring, it has been shown previously that *D. ciliatus* adults may be captured by yellow sticky traps and Mc-Phail® traps filled with *Torula* as food attractant (Deguine, Lavigne, & Atiama, 2012). Furthermore, this fly can be attracted to volatile compounds released by melon variety (*C. melo* L. cv. reticulates group) (Alagarmalai et al, 2009). Blends consisting in a mixture of four or five identified acetates were the most attractive to this fly with octanyl acetate and (Z)-3-octenyl acetate the main compounds (Alagarmalai et al, 2009).

Liquid and lure traps are tested for their efficacy against fruit flies within monitoring or mass trapping programs (Leblanc, Vargas, & Rubinoff, 2010; Lasa & Cruz, 2014; Hafsi, Abbes, Harbi, & Chermiti, 2019b; Demirel, 2019). The effectiveness of DAP© and Ceratrap© were already tested against a wide range of fruit flies worldwide. In this context, Braham (2013), highlighted the efficacy of DAP© compared to Lysatex® (commercial protein hydrolysate) in terms of trap catches against *Ceratitis capitata* Wiedemann in Tunisian citrus orchard. Various studies indicated that Ceratrap® is more efficient in decreasing fruit flies' population than other food attractants such as Starce® or proteinaceous lures (Lasa & Cruz, 2014; Hafsi et al, 2019a).

Environmental friendly control tools including attract-and-kill technique, are considered as excellent alternatives to chemical insecticides for management of fruit flies (e.g. *C. capitata*, *Anastrepha sp…)* worldwide (Piñero et al, 2009; Ryckewaert et al, 2010; Bouagga et al, 2014; Hafsi et al, 2015; Díaz-Fleischer et al, 2017). Our data indicated that trap catches decreased in Zucchini and cucumber crops where Ceranock© female and male devices were applied compared to untreated ones. Currently, no economic threshold is fixed for *D. ciliatus* management (Deguine et al, 2012). Previously, it has been shown that Nu-lure bait stations having a lifespan of 21 days, were more efficient in decreasing the number of *D. ciliatus* larvae compared to protein hydrolysate bait and insecticide treatments in white gourd crop (Qureshi

& Hussain, 1992). The authors recommended to use the Nu-lure system each three weeks for better control of *D. ciliatus* (Qureshi & Hussain, 1992). The efficacy of Ceranock® bait station was already tested against *C. capitata* in Tunisia (Bouagga et al, 2014; Hafsi et al, 2015). This device has shown effectiveness in decreasing both the number of adults (up to 70%) and fruit damages of peach orchards (Bouagga et al, 2014; Hafsi et al, 2015).

The present study provides useful data for a future application of alternative control methods against *D. ciliatus* in Tunisia. Our results confirmed the efficacy of two tested food attractants (DAP© and Ceratrap©) and attract-and-kill technique (Ceranock®) for *D. ciliatus* control in Tunisia. Ceranock® male and female showed good achievement in reducing the lesser pumpkin fly's population in cucumber and Zucchini crops. Despite the clear potential of attract-and-kill system in decreasing the number of *D. ciliatus* adults, further studies are needed to investigate the risk of this technique towards beneficial insects occurring in the field. Other researches are also required to confirm the optimum timing and duration of the application. As it is difficult, in most of cases, to eradicate an exotic fly once established, attempts for management using other effective control methods are strongly needed for food production safety.

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Composition and Structure of the Entomofauna of *Ferula* (*Ferula kuhistanica*) in Different Sections of the Zarafshan Ridge

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ABSTRACT

The article analysis the species composition of the entomofauna of the Kuhistan ferula (*Ferula kuhistanica*) in different parts of the Zarafshan ridge. The study revealed 115 species of insects belonging to 92 genera, 48 families, and eight orders. The identified species belong to the following orders: Thysanoptera (1 species), Neuroptera (3 species), Homoptera (1 species), Hemiptera (17 species), Coleoptera (36 species), Lepidoptera (5 species), Hymenoptera (14 species) and Diptera (38 species). By the nature of the relationship with the ferula, the entomofauna is divided into six ecological groups. Phytophages, including four ecological groups, accounted for 36.5% (42 species), pollinators 49.6% (57 species) and entomophagous 13.9% (16 species). A comparative analysis of the diversity of entomofauna in different parts of the Zeravshan Range was carried out, and a dendrogram of the similarity of the entomofauna of the studied territories was compiled. The horizontal and vertical isolation of the entomofauna was revealed. The most peculiar in the composition is the entomocomplex of ferula on the highest site of Saridukon. The daily activity of pollinating insects was analysed.

Keywords: Biodiversity, entomophagous, phytophagous, pollinators, Syrphidae.

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INTRODUCTION

Uzbekistan is very rich in medicinal plants, which have been widely used in folk medicine to treat many ailments since ancient times. More than 750 species of such plants grow on the republic's territory, among which representatives of the umbellifer family (Apiaceae) (119 species) prevail. At present, studies of the natural stock, cultivation on an industrial scale, and factors affecting the number and productivity of medicinal plants are gaining importance (Belolipov, Arabova, Ravshanov, & Buriyeva, 2015).

Some of the valuable medicinal plants are species of the genus Ferula. In many countries globally, various types of ferula are successfully used to treat many diseases (Iranshahy & Iranshahi, 2011; Mahendra & Bisht, 2012). In addition, *Ferula* has antioxidant (Ben Salem, Jabrane, Harzallah-Skhiri, & Ben Jannet, 2013), antiviral (Nazari & Iranshahi, 2011), antifungal (Kavoosi, Tafsiry, Ebdam, & Rowshan, 2013), and anti-diabetic (Abu-Zaiton, 2010) effects. The dried surface parts of *Ferula* (*Ferula ovina*) can be incinerated to fight the Varroa destructor mite, a dangerous parasite of bees (Shahram, Nozari, & Hosseininaveh, 2016).

Fifteen species of plants of this genus grow on the Zaravshan ridge, among which nine species are monocarpic (they bloom or bear fruit only once during their life) (Khakimzhonov, 2020). Such monocarpic species include the Ferula kuhistanica Korovin, widespread in Central Asia. This species is a perennial herb with large leaves, which are widely used as fodder and as a medicinal plant. Therefore, the demand for this plant's raw material is increasing from year to year. This led to the intensification of research on studying the plant's botanical properties and preserving its natural resources (Mukumov, Amriddinova, & Khuzhakulov, 2020).

The biological productivity of such plants largely depends on several environmental factors, among which insects are one of the most important. On the one hand, insects, as pests, cause severe damage to plants. On the other hand, pollinating insects are an essential factor in ensuring the reproduction of offspring. At present, the entomofauna of the *Ferula* is insufficiently studied. The literature data do not fully cover this issue. The available data mainly relate to the desert regions of Central Asia. In particular, VP Nevsky mentions ten species of insects closely related to plants of the stinking ferula (*Ferula assa-foetida*) in the Konimex Desert (Nevsky, 1953), and 11 species on the territory of Betpokdala (Serkova, 1958).

The entire complex of the entomofauna of the Zarafshan Range has been insufficiently studied. However, in recent years, an intensive study of individual elements of the entomofauna of this territory has been carried out, particularly syrphid flies (Rakhimov, 2021) and ground beetles (Khalimov, 2020, 2023; Zokirova & Khalimov, 2022).

Particular studies on the entomocomplex of the *Ferula assa-foetida* L. and *Ferula kyzylkumica* K. were carried out in the conditions of Southwest Kyzylkum, and more than 50 species of insects associated with these plants were identified (Davletshina & Radzivilovskaya, 1965). There are also some data on Northern

Turkmenistan and the Aydar-Arnasai lake system (Soyunov, Kamalov, & Jallieva, 1988; Avalbaev, Usanov, Umirov, & Zoirova, 2020).

However, for other regions of the world, there are some detailed studies on the role of insects in the pollination of plants from the family Apiaceae (Lindsey, 1984; Lindsey & Bell, 1985; Lamborn & Ollerton, 2000; Zych, 2002; Rovira, Bosch, Molero, & Blanche, 2004), in particular, representatives of the genera *Thaspium* and *Zizia* (Lindsey, 1984; Lindsey & Bell, 1985) and *Daucus carota* (Lamborn & Ollerton, 2000).

On the hogweed (*Heracleum sphondylium* L.), 108 species of insects were found to visit the flowers among them, medium-sized flies *Eriozona syrphoides* and *Lucilia* spp. are noted as the most effective pollinators (Zych, 2007).

The flowers of the endangered European *Ostericum palustre* Besser (Apiaceae) are visited by more than 81 anthophilous insect species and the plant is thought to be mainly pollinated by large Diptera, which are often responsible for over 90% of the total pollination (Zych, Michalska, & Krasicka-Korczyńskal, 2014). And on the flowers of Angelica sylvestris (Apiaceae), the majority of insect visits (70–91%) were made by Diptera (muscoid flies and Syrphidae) and beetles (Zych, Junker, Nepi, Stpiczynska, Stolarska, & Roguz, 2019).

MATERIAL AND METHODS

The research was carried out in 2017-2020 at 10 points of the Zarafshan ridge: Kumbelsay (39°20’ N 67°19’ E) (1400-1800 m above sea level), Saridukan pass (39°19’ N 67°11’ E) (2300-2600 m), Kamangaransay (39°22’ N 67°11’ E) (1500-2000 m), Sariktepasay (39°21’ N 67°07’ E) (1400-1900 m), Ettuilisay (39°26’ N 66°59’ E) (1100-1300 m), Takhtakaracha pass (39°18’ N, 66°53’ E) (1700-2000 m); Amankutan (39°18’ N 66°57’ E) (1400-1500 m), Ayrikoya village (39°18’ N, 66°53’ E) (1400-2000 m), Agalyksay (39°27’ N 66°49’ E) (1000-1900 m) (northern slope of the ridge) and Bashyr (39°16’ N 67°06’ E) (1000-1200 m) (southern slope of the ridge) (Fig. 1).

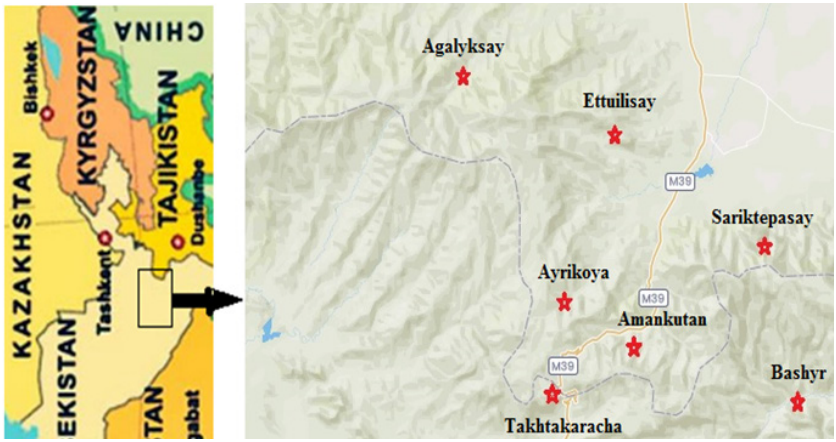

Figure 1. Map of the research area.

The study was carried out in 2018-2021 during the growing season of *F. kuhistanica* (from March to August). Materials were collected using general entomological methods: larger and less active insects were collected manually, agile, fast-flying insects were collected with an entomological net, and small insects were collected with an exhauster. During the collection, the lifestyle and behavior of individuals of significant species were studied.

To study the role of pollinators, a quantitative analysis of insects that arrived at the plants was carried out. Accounting was carried out on three plots of different heights (Ettiuilisay, Takhtakaracha and Saridukon) on different dates depending on the ferula flowering period (3 times: at the beginning, at the peak and at the end of flowering). Recording dates: at the Ettiuilisay site - 25.03.2019, 9.04.2019, 8.05.2019; at the Takhtakaracha section - 1.04.2019, 14.04.2019, 13.05.2019; at the Saridukon site on 15.05.2019, 1.06.2019, 1.07.2019. The counts were carried out three times a day (9:00, 12:00 and 17:00).

To study the daily activity of syrphid flies for 10 minutes at the beginning of each hour of the day, the visit of syrphid to ferula flowers was taken into account. The counts were carried out during the period of mass flowering of plants from 6:00 to 18:00 days in three repetitions. The results of these counts are presented in Figure 2.

A comparison of the entomocomplex of the studied areas was carried out based on the Chekanovsky-Sørensen coefficient (Dunaev ,1997). The Chekanovsky-Sørensen coefficient was calculated using the formula $Cs = 2j / (a + b)$, where: *Cs*-Chekanovsky-Sørensen coefficient; *j*- is the number of species common to two biotopes; *a* and *b* - the number of species in the compared biotopes.

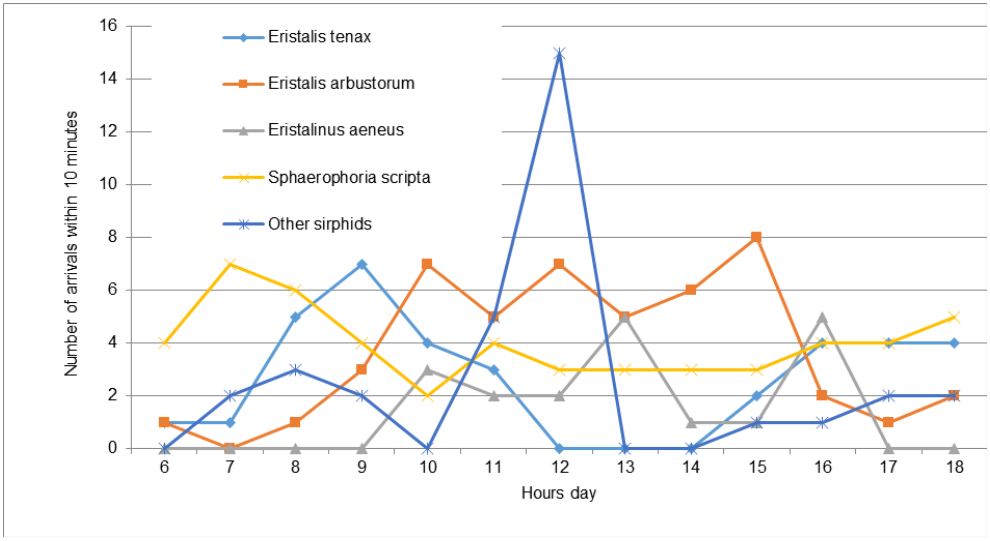

Figure 2. Diurnal dynamics of Syrphidae on *Ferula kuhistanica*.

RESULTS AND DISCUSSION

One hundred fifteen species of insects have been identified that are somehow associated with the ferula. The identified species belong to 8 orders: Thysanoptera (1 species), Neuroptera (3 species), Homoptera (1 species), Hemiptera (17 species), Coleoptera (36 species), Lepidoptera (5 species), Hymenoptera (14 species), and Diptera (38 species). We conditionally divided these insects into three ecological groups, depending on their relationship with the ferula: phytophages (feeding on different parts of plants), pollinators, and entomophagy (Table 1). It should be noted that many pollinators are phytophages, but their harm is not perceptible to plants (Fengri & van der Peil, 1982).

Table 1. Species composition of the entomofauna *Ferula kuhistanica* L.

Ordo	Family	Species
PHYTOPHAGES FEEDING ON ROOTS AND STEM		
Hemiptera	Pentatomidae	*Carpocoris purpureipennis* (De Geer, 1773)
	Thripidae	*Tenothrips frici* Uzel, 1895
Coleoptera	Scarabaeidae	*Protaetia (Netocia) turkestanica* (Kraatz, 1886)
	Buprestidae	*Anthaxia anatolica lucidiceps* Gory 1841
		Anthaxia plavilschikovi Obenb. 1935
	Cerambycidae	*Plocaederus scapularis* Fischer, 1821
	Curculionidae	*Cyphocleonus tigrinus* (Panzer, 1789)
		Mecaspis alternans (Herbst, 1795)
		Lixus capiomonti Faust, 1883
LEAF-FEEDING PHYTOPHAGOUS		
Homoptera	Aphididae	*Dysaphis* sp.
	Pentatomidae	*Antheminia lunulata* (Goeze, 1778)
		Dolycoris penicillatus Horvath, 1904
		Dolycoris varicornis montandoni Sienkiewicz, 1954
Hemiptera	Miridae	*Dicyphus orientalis* Reuter, 1879
		Orthop campestris (Linnaeus, 1758)
	Tingitidae	*Tingis cardui* Linnaeus, 1758
	Myodochidae	*Lygaeus equestris* (Linnaeus, 1758)
	Coreidae	*Corpus* sp.
Coleoptera	Chrysomelidae	*Ichyronota conicicollis* Weise, 1890
Lepidoptera	Nymphalidae	*Melitaea acareina* Staudinger, 1886
	Noctuidae	*Autographa gamma* (Linnaeus, 1758)
FLOWER-EATING PHYTOPHAGOUS		
Hemiptera	Pentatomidae	*Grafosoma lineolatum* (Linnaeus, 1758)
Coleoptera	Scarabaeidae	*Oxythyrea cinctella* (Schaum, 1841)
		Cetonia trojan Gory & Percheron, 1833
	Staphylinidae	*Omalium rivulare* (Payk., 1789)
		Stenus sp.
	Nitidulidae	*Meligethes* sp.
	Meloidae	*Mylabris frolovi* Germar, 1824
		Mylabris magnoguttata (Heyden, 1881)
		Meloe violaceus Marsham, 1802
		Teratolytta pilosella (Solsky, 1881)
		Cerocoma schreberi (Fabricius, 1781)
		Rhampholyssa antennata Reitter, 1906
		Aloysius syriacus (Linnaeus, 1758)

Ordo	Family	Species
Coleoptera	Alleculidae	*Omophlus curtus* Kuster, 1850
		Omophlus deserticola (Kirsch, 1869)
	Mordellidae	*Mordella aculeata* Linnaeus, 1758
	Prionoceridae	*Lobonyx* sp.
	Cerambycidae	*Agapanthus soror* Kraatz, 1882
	Elateridae	*Lacon funebris* (Solsky, 1881)
SEED-FEEDING PHYTOPHAGOUS		
Hemiptera	Myodochidae	*Ryparochromus quadratus* (Fabricius, 1798)
	Coreidae	*Camptopus lateralis* (Germar, 1817)
POLLINATORS		
Coleoptera	Cantharididae	*Cantharis forticornis* Heyden, 1885
		Cantharis livida Linnaeus, 1758
	Dermestidae	*Paranovelsis quadricolor* (Sumakov, 1907)
		Attagenus pictus Ballion, 1871
	Cleridae	*Trichodes axillaris* Fischer de Waldheim, 1842
	Melyridae	*Malachius bipustulatus* (Linnaeus, 1758)
Lepidoptera	Lycaenidae	*Tomares callimachus* (Eversmann, 1848)
	Nymphalidae	*Argynnis paphia* (Linnaeus, 1758)
		Satyrus sp.
Hymenoptera	Crabronidae	*Crabro albilabris* Fabricius, 1793
		Ectemnius fossorius (Linnaeus, 1758)
	Andrenidae	*Andrena carbonaria* (Linnaeus, 1767)
		Andrena sp.
	Megachilidae	*Megachile apicalis* Spinola 1808
		Anthidium sp.
		Coelioxys sp.
	Pompilidae	*Agenioideus apicalis* (Vander Linden, 1827)
	Vespidae	*Polistes dominula* (Christ, 1791)
	Apidae	*Anthophora semperi* Fedtschenko 1875
	Halictidae	*Halictus* sp.
Diptera	Syrphidae	*Episyrphus balteatus* (De Geer, 1776)
		Eupeodes corollae (Fabricius, 1794)
		Eupeodes nuba (Wiedemann, 1830)
		Scaeva albomaculata (Macquart, 1842)
		Scaeva latimaculata (Brunetti, 1923)
		Scaeva pyrastri (Linnaeus, 1758)
		Spaerophoria scripta (Linnaeus, 1758)
		Sphaerophoria rueppellii (Wiedemann, 1830)
		Syrphus vitripennis Meigen, 1822
		Xanthogramma hissarica Violovitsh, 1975
		Chrysotoxum bacterium Violovitsh, 1973
		Chrysotoxum vernale Loew, 1841
		Melanostoma mellinum Linnaeus, 1758
		Platycheirus ambiguus Fallén, 1817
		Paragus bicolor (Fabricius, 1794)
		Paragus haemorrhous Meigen, 1822
		Paragus tibialis (Fallén, 1871)
		Paragus quadrifasciatus Meigen, 1822
		Pipizella mesasiatica Stackelberg, 1952
		Cheilosia aerea Dufour 1848

Ordo	Family	Species
Diptera	Syrphidae	*Cheilosia lola* Zimina, 1970.
		Cheilosia stackelbergi Barkalov & Peck, 1994
		Chrysogaster musatovi Stackelberg, 1952
		Chrysogaster tadjikorum Stackelberg, 1952
		Eumerus aristatus Peck, 1969
		Eumerus coeruleus (Becker, 1913)
		Eumerus kondarensis Stackelberg, 1952
		Eumerus pamirorum Stackelberg, 1949
		Eumerus ursiculus Stackelberg, 1949
		Merodon tarsatus Sack, 1913
		Eristalis (Eoseristalis) arbustorum (Linnaeus, 1758)
		Eristalis (Eristalis) tenax (Linnaeus,1758)
		Myathropa semenovi (Smirnov, 1925)
		Syritta pipiens (Linnaeus, 1758)
	Bibionidae	*Bibio hortulanus* (Linnaeus, 1758)
	Scatophagidae	*Scatophaga stercoraria* Linnaeus, 1758
	Calliphoridae	*Calliphora erythrocephala* Macquart, 1834
ENTOMOPHAGOUS		
Hemiptera	Anthocoridae	*Orius niger* Wolff, 1841
	Nabidae	*Nabis maracandicus* Reuter, 1890
		Nabis palifer Seidenstucker, 1954
	Reduviidae	*Rhynocoris iracundus* (Poda, 1761)
		Coranus aegyptius (Fabricius, 1775)
Neuroptera	Ascalaphidae	*Ascalaphus macaronius* (Scopoli, 1763)
	Chrysopidae	*Chrysopa vulgaris* Schneider, 1851
		Chrysopa abbreviate Curtis, 1834
Coleoptera	Coccinellidae	*Coccinella septempunctata* Linnaeus, 1758
		Hippodamia variageta (Goeze, 1777)
		Adalia bipunctata (Linnaeus, 1758)
	Carabidae	*Poecilus liosomus* Chaudoir,1876
Hymenoptera	Braconidae	*Microplitis spinolae* (Nees, 1834)
	Ichneumonidae	*Ophion luteus* (Linnaeus, 1758)
	Sphecidae	*Sphex* sp.
Diptera	Asilidae	*Satanas gigas* (Eversmann, 1855)

As the results show, the species diversity of the ferula entomocomplex on different parts of the ridge, depending on the biotope and altitude above sea level, differs significantly (Table 2). The most diverse species composition is the biotopes of Amankutan (1400-1500 m above sea level) (82 species). The main reason for this diversity is, most likely, the hydrological regime of the area, since these biotopes are the most hydrated compared to other biotopes. The smallest diversity of the ferula entomofauna was noted in the biotopes of Sariktepasai (1400-1900 m) and Airikoya (1400-2000 m) (37 species each).

As reported in the literature, the formation of entomfauna depends on both the vertical and horizontal isolation of biocenoses. To find out which of them is primary in the formation of the entomocomplex of the ferula, we grouped the studied biotopes by height and latitude. Three zones were distinguished by height: low (1000-1400 m above sea level), medium (1400-2000 m above sea level), and high

(2000-2600 m above sea level). The following are identified horizontally: Northern Chakalikalyan (sections Kumbelsay, Saridukon, Kamangaransay, Sariktepasay), Karatepa (sections Takhtakaracha, Amankutan, Airikoya, Ettiuilisay and Agalyksay) and South Chakalikalyan (section Bashyr). We proceeded from the fact that if the entomocomplex of the ferula will differ to a greater extent in vertical zones, then in the formation of the entomocomplex of the ferula of the Zarafshan ridge, vertical zoning is more pronounced, and if the entomocomplex of the ferula will differ to a greater extent in the horizontal zones, then the formation of the entomocomplex of the ferula is characteristic of (mosaic). Conventionally considering each site as one biotope, the Chekanovsky-Sørensen coefficient was used for a comparative analysis of entomocomplexes (Table 3).

Table 2. Diversity of the ferula entomocomplex (number of species) in different parts of the Zarafshan ridge (The relative abundance of species is calculated on the basis of the proportion of species of a particular point from the total number of species).

The main components of the entomofauna	Total	Kumbelsay	Saridukan pass	Kamangaransay	Sariktepasay	Ettuilisay	Takhtakaracha pass	Amankutan	Ayrikoya village	Agalyksay	Bashyr
Phytophages	42	21	26	25	18	21	32	36	22	29	33
Pollinators	57	15	36	18	14	19	28	37	11	21	29
Entomophagy	16	6	9	5	5	5	6	9	4	4	9
Total species	115	42	71	48	37	45	66	82	37	54	71
Relative abundance of species,%	100	36.5	61.7	41.7	32.2	39.1	57.4	71.3	32.2	47.0	61.7

Table 3. Similarity coefficient of the ferula entomocomplex at ten sites of the Zarafshan Range (Chekanovsky-Sørensen coefficient / number of common species).

Sites	Kumbelsay	Saridukan pass	Kamangaransay	Sariktepasay	Ettuilisay	Takhtakaracha pass	Amankutan	Ayrikoya village	Agalyksay	Bashyr
Kumbelsay		25	29	25	23	33	39	20	21	24
Saridukan pass	0.44		29	25	20	38	41	21	23	23
Kamangaransay	0.64	0.49		35	25	32	43	23	16	28
Sariktepasay	0.63	0.46	0.82		26	27	30	20	21	25
Ettuilisay	0.53	0.35	0.54	0.63		39	44	26	35	22
Takhtakaracha pass	0.61	0.56	0.56	0.52	0.70		56	35	31	34
Amankutan	0.63	0.54	0.66	0.50	0.69	0.76		36	30	33
Ayrikoya village	0.51	0.39	0.54	0.54	0.63	0.68	0.61		32	25
Agalyksay	0.44	0.37	0.31	0.46	0.71	0.52	0.44	0.70		19
Bashyr	0.43	0.32	0.47	0.46	0.38	0.50	0.43	0.46	0.30	

The analysis showed that the most remarkable similarity of the entomofauna of the ferula is observed between the sites of Sariktepasai and Kamangaransay (0.82) and between the Takhtakaracha Pass and Amankutan (0.76). The entomofauna of the Bashyr site is peculiar since the least similarity was observed here compared to the sites Agalyksay (0.30) and Saridukon Pass (0.32). For clarity of the results obtained, based on the Chekanovsky-Sørensen coefficient, a dendrogram was drawn up (Fig. 2).

Analyzing the obtained data, it can be concluded that for the formation of the entomocomplex of the ferula, horizontal zoning is more important than vertical zoning. Thus, the areas of Northern Chakalikalyan (Kumbelsai, Kamangararansay, Sariktepasai) are similar in the composition of the entomofauna (the exception is the Saridukon area), while the Karatepa areas (Takhtakaracha, Amankutan, Airikoya, Ettiuilisay and Agalyksai differ significantly from them. The entomocomplex of the ferula of South Chakalikalyan (Bashyr) is isolated. However, one cannot ignore the fact that vertical zoning also significantly affects the formation of entomofauna. So, for example, the ferula entomocomplex at the highest research site (Saridukon) turned out to be the most peculiar and significantly differs even from the neighboring areas of Northern Chakalikalyan (Fig. 2).

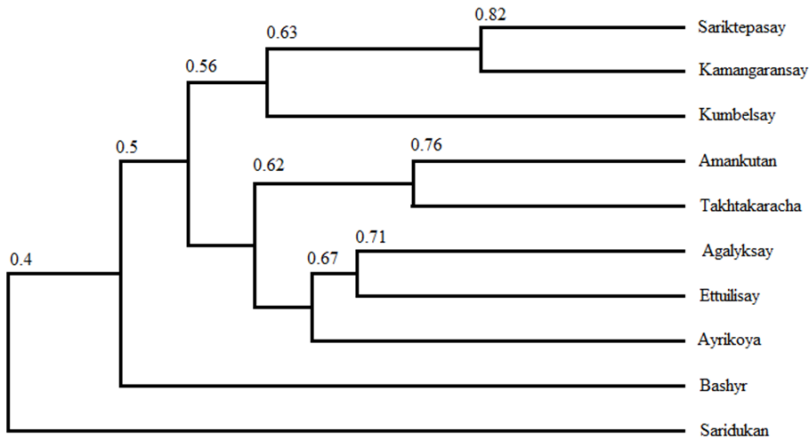

Figure 2. Dendrogram of the similarity of the ferula entomocomplex in different parts of the Zarafshan Range, built using the UPGMA method based on the Czekanowski-Sørensen coefficient.

In recent years, as mentioned above, in the Republic of Uzbekistan, due to the significant interest in medicinal plants, there has been an expansion of ferula crops on agricultural land in the foothills (Republic of Uzbekistani, 2020). In this regard, many questions arise on the cultivation and cultivation of this valuable medicinal plant, one of which is the need to study saw-sawing insects, which play an essential role in seed reproduction.

To elucidate the activity of individual groups of insects in the pollination of ferula flowers, we selected three stationary sites (Ettiuilisay, Takhtakaracha, and Saridukon). In these plots, three times a day (900, 1200, and 1700) for 30 minutes, the number of insects that arrived or were on the flowers of the ferula were caught and counted. The experiments were carried out three times per season: at the beginning of flowering plants, after two weeks, and after 45 days. Although the results will be relative, they may well be suitable for comparing the number and activity of different pollinating insects (Faegri & van der Pijl, 1982).

The results show that representatives of the families Crabronidae and Megachilidae from the order Hymenoptera and the family Syrphidae from the order Diptera are numerous in all research areas. Together, these three families make up 60% of all pollinators (Table 4).

Table 4. The composition and activity of pollinating insects in the Kuhistan ferula (the number of insects that arrived every 30 minutes)As can be seen from the table, the number of pollinating insects decreases in the order of Hymenoptera - Diptera –Coleoptera –Hemiptera –Lepidoptera.

Main groups of pollinators		Research sites						A total of three sites	
		Ettiuilisay		Takhtakaracha		Saridukon			
		number of visits	%	number of visits	%	number of visits	%	number of visits	%
Hemiptera		0.67	0.98	5.0	4.75	0.67	2.11	6.33	3.08
Coleoptera		3.33	4.9	16.7	15.8	3.33	10.5	23.3	11.4
Lepidoptera		0.33	0.49	1.67	1.58	2.0	6.33	4.0	1.95
Diptera	Syrphidae	9.0	13.2	9.33	8.86	14.7	46.3	33.0	16.1
	others	3.33	4.9	6.67	6.33	8.33	26.4	18.33	8.94
Hymenoptera	Crabronidae	21.7	31.9	33.0	31.3	1.67	5.26	56.3	27.5
	Megachilidae	11.3	16.7	22.3	21.2	-	-	33.7	16.4
	Andrenidae	6.67	9.8	2.0	1.9	-	-	8.67	4.23
	others	11.7	17.2	8.67	8.23	1.0	3.16	21.3	10.4
Total		68.0	100	105.3	100	31.7	100	205.0	100

However, it should be noted that the effectiveness of pollinators depends not only on their number but also on their behavior. For example, due to the absence or paucity of hairs on the body, many bugs and beetles are of little importance in plant pollination (Faegri & van der Pijl, 1982).

The ferula has several inherent characteristics that create the conditions for the effectiveness of many pollinators. Firstly, the perianth of the ferula is not very deep, which facilitates access to nectar, especially for many dipterans. Secondly, the flowers of the ferula are yellow light, which is attractive to many insects. Another feature of the ferula is its smell, which attracts pollinators, saprophages, and necrophages. Therefore, on the ferula, you can always find many different insects.

Pollinator activity changes significantly during the day. We have studied the daily activity of pollinating insects using the example of Diptera species from the family Syrphidae (Fig. 2).

Studies show that different types of syrphids are active at different times of the day. For example, the species Sphaerophoria scripta and Eristalis tenax are more active in the morning and afternoon. The Eristalis arbustorum species is most active from 10:00 to 15:00 hours. In general, many species of hoverflies are most active by 12:00 hours of the day. The activity of the ferula pollinators is significantly influenced by illumination, temperature, and wind speed, which requires special additional study.

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New Records of Ceutorhynchinae (Insecta: Coleoptera, Curculionidae) for the Fauna of Turkish Thrace and Türkiye

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ABSTRACT

New records for Türkiye of Ceutorhynchinae weevils are reported. *Poophagus sisymbrii* (Fabricius, 1977) is reported for the first time from Türkiye. In addition, *Datonychus melanostictus* (Marsham, 1802), *Pelenomus commari* (Panzer, 1795) and *Rhinonchus bruchoides* (Herbst, 1784), already known from the Anatolian part of Türkiye, are recorded for the first time from Turkish Thrace. Additional localities of a few species already known from Thrace region are also given.

Keywords: New records, ceutorhynchines, *Poophagus*.

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INTRODUCTION

Currently, 270 species of the subfamily Ceutorhynchinae Gistel, 1848 are recorded from Türkiye (Gültekin, 2014; Aydın & Hacet, 2016a; Korotyaev et al, 2017; Hacet & Colonnelli, 2019; Alonso-Zarazaga et al, 2023). Of these, 55 species have been recorded from Turkish Thrace, which is the European part of Türkiye (Hacet & Colonnelli, 2019; Alonso-Zarazaga et al, 2023).

Of the six tribes of Ceutorhynchinae (Amalini Wagner, 1936, Ceutorhynchini Gistel, 1848, Mononychini LeConte, 1876, Phytobiini Gistel, 1848, Hypurini A. Schultze, 1902 and Scleropterini A. Schultze, 1902) recorded in Türkiye, Ceutorhynchini and Phytobiini are represented by the greatest number of species, as in the rest of the world. The absolute majority of ceutorhynchines are terrestrial, excepting a few species of Ceutorhynchini and several ones of Phytobiini which are aquatic or semi-aquatic (Morris, 2008). Aquatic habitats are nowadays places where pollution is fast spreading over large areas. For more effective conservation studies and for understanding consequences of climate change on the distribution of living distribution, ecosystem components should be determined as soon as possible. Therefore, wetlands are interesting habitats to explore also for Ceutorhynchinae.

In this note are reported four semi-aquatic species, one of them previously unrecorded from both Asian and European parts of Türkiye. This last belongs to the genus *Poophagus* Schoenherr, 1837 which was thus far unknown from the whole of Türkiye. Three other ones are recorded for the first time from the European part of Türkiye.

MATERIAL AND METHODS

This note is mainly based on material collected by the first author and Meral Fent in Turkish Thrace in 2016, 2018, 2019 and 2020. Samples were obtained by using sweeping net especially from plants growing on streamside. Specimens were initially preserved in 70% ethyl alcohol, and then they were mounted on tips of triangular labels and labelled. The material is preserved in the Zoological Museum in the Biology Department of Trakya University, Edirne, Türkiye. Collecting locations in Turkish Thrace are shown in Fig.1. Species are cited by tribe in alphabetical order, and also distribution and a short description are given for the newly recorded ones.

Localities

Edirne province. Loc. 1: Meriç-Olacak, 18.5.2016, 47 m, 41°12.55'N 26°28.25'E. Loc. 2: Kırcasalih-Tahal (Suvat Lake), 8.6.2020, 63 m, 41°25.868'N 26°51.764'E. Loc. 3: Süloğlu-Sülecik, along a stream, 12.6.2020, 196 m, 41°48.908'N 26°50.700'E.

Kırklareli province. Loc. 4: Soğucak, streamside, 25.5.2018, 271 m, 41°38.07'N 27°39.23'E. Loc. 5: Pınarhisar-Poyralı, stream, 25.5.2018, 246 m, 41°37'47'N 27°35.51'E. Loc. 6: Center-Koyunbaba, stream, 12.6.2020, 152 m, 41°43.588'N 27°6.048'E. Loc. 7: Ürünlü, streamside, 7.7.2019, 115 m, 41°40.430'N 26°59.478'E.

İstanbul province. Loc. 8: Mimar Sinan, streamside, 20.6.2019, 60 m, 41°9.448'N 28°55.260'E. Loc. 9: Çatalca entrance-Karasu stream, 3.7.2019, 62 m, 41°10.378'N 28°26.204'E. Loc. 10: Çatalca-İnceğiz bridge, stream, 4.7.2019, 39 m, 41°10.938'N 28°24.216'E. Loc. 11: road to Danamandıra from Çatalca, streamside, 10.7.2019, 232 m, 41°19.327'N 28°15.948'E.

Tekirdağ province. Loc.12: Malkara-Kozyörük, side of Güneşli brook, 15.6.2020, 120 m, 41°0.133'N 26°56.557'E.

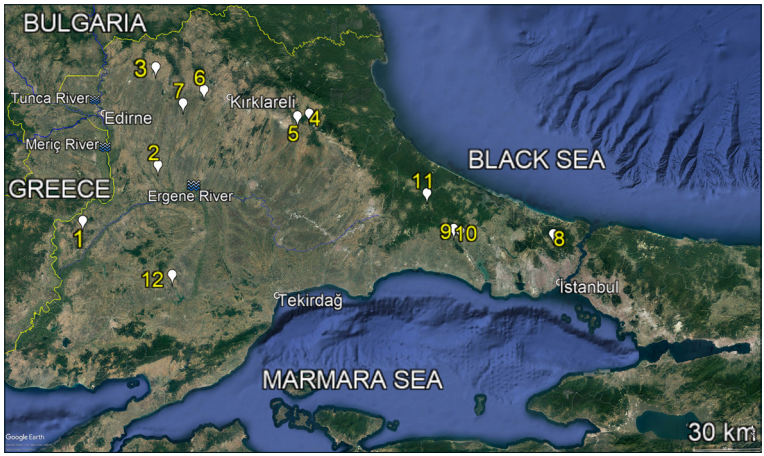

Figure 1. Localities, the study material was sampled in Turkish Thrace. The numbers correspond to the localities (from Google Earth).

RESULTS

Ceutorhynchini Gistel, 1848

*Amalorrhynchus melanarius (*Stephens, 1931)

Material examined. İstanbul province: Loc. 9, 1 ♂.

Comments. Species known only from one locality in Türkiye at the present (Hacet & Colonnelli, 2019). This second record is from a different locality of the İstanbul province. *Amalorrhynchus melanarius* is to be found in semiaquatic habitats (Morris, 2008), and the collected single specimen was swept from plants growing on the banks of a stream.

Ceutorhynchus pallidactylus (Marsham, 1802)

Material examined. Edirne province: Loc. 2, 1 ♀; Tekirdağ province: Loc. 12, 2 ♂♂, 1 ♀.

Comments. This species has been previously reported from Edirne, İstanbul and Kırklareli provinces in the Turkish Thrace (Hacet & Colonnelli, 2019). This is an additional locality of the Edirne province, and a new record for Tekirdağ province.

***Ceutorhynchus viridipennis* C.Brisout de Barneville, 1869**

Material examined. Edirne province: Loc. 3, 1 ♂.

Comments. This species has been reported from Edirne and Çanakkale provinces in the Turkish Thrace (Hacet & Colonnelli, 2019). This is an additional locality of the Edirne province.

***Datonychus melanostictus* (Marsham, 1802)**

Material examined. Kırklareli province: Loc. 6, 1 ♀.

Distribution. Europe: Austria, Belgium, Bulgaria, Czech Republic, Denmark, England, France, Germany, Greece, Hungary, Italy, Liechtenstein, Luxembourg, Moldavia, The Netherlands, Poland, Portugal, Romania, Serbia, Slovakia, South European Russia, Spain, Sweden, Switzerland, Ukraine. North Africa: Algeria, Canary Islands, Morocco. Asia: Armenia, Azerbaijan, Georgia, Iran, Jordan, Kyrgyzstan, Syria, Turkmenistan, Türkiye, Uzbekistan (Alonso-Zarazaga et al, 2023).New record for Turkish Thrace.

Short description. Body elongate, about 3 mm long. Integument piceous, tarsi reddish. Dorsal side covered with brownish and whitish scales on pronotum and elytra, here forming an arrow-like pattern on basal third, the arms of the arrow pointing forward towards humeri, plus a quite confused pale band on elytral posterior third. Underside clothed by pale greyish scales. Femora toothed, tarsal claws appendiculate.

Comments. No records of any *Datonychus* Wagner, 1944 were known from Turkish Thrace up to present. Wetlands, and wet areas even inside agricultural areas are habitats where the species occurs on the Lamiaceae *Lycopus europaeus* L. and *Mentha* spp. (Colonnelli, 2004; Morris, 2008), both genera of plants are recorded from the Turkish Thrace (Tübives, 2022).

***Hadroplontus trimaculatus* (Fabricius, 1775)**

Material examined. Kırklareli province: Loc. 4, 1 ♀.

Comments. This species has been reported from the Edirne province in the Turkish Thrace (Aydın & Hacet, 2016b; Hacet & Colonnelli, 2019). There is another record from Kırklareli province (Lodos et al, 1978), the present one is from a new locality in the Kırklareli province after some 40 years.

***Microplontus rugulosus* (Herbst, 1795)**

Material examined. Edirne province: Loc. 1, 2 ♀♀.

Comments. Species already known from the Edirne province in the Turkish Thrace (Aydın & Hacet, 2016b; Hacet & Colonnelli, 2019). This is an additional locality of the same province.

***Mogulones geographicus* (Goeze, 1777)**

Material examined. Kırklareli province: Loc. 5, 1 ♂.

Comments. This species has been registered from the Edirne province in the Turkish Thrace by Aydın & Hacet (2016b) and Hacet & Colonnelli (2019). This is a new locality for the Kırklareli province.

Poophagus sisymbrii (Fabricius,1777)

Material examined. İstanbul province: Loc.9, 1 ♀.

Distribution. Europe: Austria, Belgium, Belarus, Bulgaria, Croatia, Czech Republic, Denmark, England, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Luxembourg, Moldavia, The Netherlands, Poland, Romania, Central and South European Russia, Slovakia, Slovenia, Sweden, Ukraine. Asia: Kazakhstan, East Siberia, Russian Far East, West Siberia. Nearctic Region: Canada (Colonnelli, 2004; Alonso-Zarazaga et al, 2023). New record for Türkiye.

Short description. Body elongate (collected sample: 3.2 mm), black, covered with grey scales on head, pronotum, elytra, legs, and underside of body. Antennae with 7-segmented funicle and scape gradually expanded from the midpoint. Central area of pronotum, disc of elytra and two patches below shoulders dark. Tibiae unarmed, tarsal claws simple.

Comments. The genus includes three species, all from the Palearctic (Alonso-Zarazaga et al, 2023). Of them, *P. hopffgarteni* Tournier, 1873 occurs from central-eastern Europe to West Siberia, and *P. robustus* Faust, 1881 is distributed in Eastern Europe, Kazakhstan and West Siberia. The distribution of *P. sisymbrii* includes almost all the Palearctic Region, being its presence in the Nearctic Region (Quebec, Canada) due to an involuntary introduction (O'Brien & Wibmer, 1982) from Europe. *Poophagus sisymbrii* is related to wetlands, occurring at the side of streams, rivers and canals (Morris, 2008). Host plants of this species are as the genera *Nasturtium* W.T. Aiton and *Rorippa* Scop. of Brassicaceae (Colonnelli, 2004; Morris, 2008). The specimen collected in Turkish Thrace was swept by plants growing at the side of a brook, but its host was not identified. *Nasturtium officinale* W.T. Aiton, *Rorippa austriaca* (Crantz) Besser, *R. thracica* (Gris.) Fritsch, and *R. sylvestre* (L.) Bess. are recorded from İstanbul province (Tübives, 2022), possibly being one or more of them the host of *P. sisymbrii* in Türkiye. The finding in the province of İstanbul represents the easternmost record of the species in southeastern Europe.

Phytobiini Gistel, 1848

Pelenomus commari (Panzer, 1795)

Material examined. Kırklareli province: Loc. 7, 2 ♀♀.

Distribution. Europe: Austria, Belarus, Belgium, Bosnia Herzegovina, Bulgaria, Czech Republic, Denmark, England, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Luxembourg, Moldavia, The Netherlands, Norway, Poland, Romania, European Russia, Serbia, Slovakia, Slovenia, South European Russia, Spain, Sweden, Switzerland, Ukraine. Asia: Kazakhstan, Türkiye, West Siberia (Alonso-Zarazaga et al, 2023). New record for Turkish Thrace.

Comments. Although four species (including *P. commari*) of the genus *Pelenomus* C. G. Thomson 1859 are reported from Türkiye, there is previously any record of the genus from the Turkish Thrace (Hacet & Colonnelli, 2019; Alonso-Zarazaga et al, 2023). The habitats in which the species occur are wetlands, as in *Amalorrhynchus melanarius* and *Poophagus sisymbrii* recorded above. Turkish Thrace is the easternmost record of *P. commari* in southeastern Europe. The specimen was collected sweeping plants at the the side of a brook. The Rosaceae *Potentilla palustris* L., *Sanguisorba officinalis* L., *Alchemilla vulgaris* L., and the Lythraceae *Lythrum salicaria* L. are plants where *P. commari* usually occurs (Colonnelli, 2004; Morris, 2008).

***Rhinonchus bruchoides (*Herbst, 1784)**

Material examined. Kırklareli province: Loc. 7, 1 ♂; İstanbul province: Loc. 8, 25 ♂♂, 20 ♀♀; Loc. 10, 1 ♀.

Distribution. Europe: Austria, Belarus, Belgium, Bulgaria, Czech Republic, Denmark, England, Estonia, France, Germany, Greece, Hungary, Italy, Latvia, Luxembourg, Moldavia, The Netherlands, Norway, Poland, Portugal, Romania, European Russia, Serbia, Slovakia, Slovenia, South European Russia, Spain, Sweden, Switzerland, Ukraine. Asia: northern China, Georgia, Japan, Kazakhstan, Mongolia, East Siberia, Russian Far East, Türkiye, West Siberia. Nearctic Region: United States (Colonnelli, 2004; Alonso-Zarazaga et al, 2023). New record for Turkish Thrace.

Comments. Two species of the genus *Rhinoncus* Schoenherr, 1925 are known from Turkish Thrace up to present (Hacet & Colonnelli, 2019). *Rhinoncus bruchoides* is recorded for the first time from the region. This species usually occurs in wedlands, where its host plants are *Polygonum lapathifolia* L. and *P. hydropiper* L. (Morris, 2008). Both above species of Polygonaceae are recorded from İstanbul province, and furthermore *Polygonum salsugineum* Bieb. is known from Kırklareli province (Tübives, 2022).

***Rhinoncus perpendicularis (*Reich, 1797)**

Material examined. Kırklareli province: Loc. 7, 4 ♂♂, 4 ♀♀; İstanbul province: Loc. 11, 1 ♀.

Comments. This species has been reported from Edirne province in the Turkish Thrace (Aydın & Hacet, 2016b). Both the above localities where this weevil occurs are other provinces located in the European part of Türkiye.

DISCUSSION

With the record of *Poophagus* the number of Ceutorhynchinae genera increases to 48 from 47 in Türkiye. Furthermore, the genera number known from Turkish Thrace increases to 24 from 21 with the finding of a *Datonychus*, a *Poophagus* and a *Pelenomus,* and the species number is now 59 with four new records for the region (*Datonychus melanostictus*, *Poophagus sisymbrii*, *Pelenomus commari* and *Rhinoncus bruchoides*). The paucity of records of wetland species is probably due to underexploration of these habitats, well worthy of further investigations in the future.

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New a Species as Parasitoid of the Apple Ermine Month *Yponomeuta malinellus* Zeller, 1838 (Lepidoptera: Yponomeutidae) in the Çoruh Valley, Erzurum Province, Türkiye

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ABSTRACT

The current study aimed to determine parasitoids of *Yponomeuta malinellus* Zeller, 1838 (Lepidoptera: Yponomeutidae) in apple trees in the Çoruh Valley, Erzurum Province, Türkiye during 2019-2020 summer. The parasitoids associated with *Y. malinellus* were reared in a laboratory, with a total of 137 individual parasitoids emerging from family Ichneumonidae (Hymenoptera). Among the identified species *Trieces facialis* (Thomson, 1887), *Itoplectis tunetana* (Schmiedeknecht, 1914) and *Pimpla turionellae* (Linnaeus, 1758) were determined as pupal parasitoid. Of these, *Trieces facialis* has been obtained from *Y. malinellus* our country first time and also is new for the Turkish Ichneumonidae fauna. In this study of the Çoruh Valley, the mean parasitism rate was %27.4.

Keywords: *Yponomeuta malinellus,* apple, parasitoid, Çoruh Valley, Türkiye.

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INTRODUCTION

Yponomeuta malinellus Zeller, 1838 (Lepidoptera: Yponomeutidae), the apple ermine month is native throughout the temperate zone of the Palaearctic region (Balachowsky, 1966).

Yponomeuta malinellus is belonging to order Lepidoptera and family Yponomeutidae (Gershenzon, 1986). "The larvae of pest vary as they grow from dark grey to yellowish grey, and the size of black spots changes. Full grown larvae vary from 18 to 25 mm. The white cocoons are arranged neatly side by side in a web beneath a leaf or branch of tree (CFIA, 2006).

The adult has pure white fore wings (sometimes with a slight grey suffusion at the centre), with black dots. The terminal cilia are normally slightly grey but can be pure white. It has a white head with white palps (CFIA, 2006).

The larvae of the apple ermine moth, gnawing leaves. It can defoliate apple trees and negatively impact fruit production for several years following an outbreak (Anonymous, 2020).

This pest has spread all over the world (Azerbaijan, Armenia, Canada, China, Czech Finland, France, Georgia, Germany, Kazakhstan, Iran, Italy, Korea, Japan, Lithuania, the Netherlands, Pakistan, Sweden, Turkey, Ukraine, Uzbekistan and the United Kingdom (Gershenzon, 1970; Pustovarov, 1980; Mamedov & Makhmudova-Kurbanova, 1982; Arduino, Cianchi, & Bullini, 1983; Kuhlmann, Carl, & Mills, 1988; Unruh, Congdon, & La Gasa, 1993; Jonaitis, 2001; Gençer, 2003; Hrudová, 2003; Lee & Pemberton, 2005; CFIA, 2006; Kimber, 2011; Alaserhat, 2019). .

The pest's parasitoids have attracted the attention of many researchers. Many studies have been conducted in the former Soviet Union and other countries, especially Europe (Beirne, 1943; Junnikkala, 1960; Friese, 1963; Affolter & Carl 1986; Dijkerman, Groot, & Herrebout, 1986; Kuhlmann, 1996). There are studies on the pest in our country, but it is not sufficient (İren, 1960; Koçak, 1989; Bulut & Kılınçer 1989; Erol & Yaşar, 1996; Tozlu, Özbek, & Gültekin, 2000; Gençer, 2003; Çoruh, 2005; Çoruh & Özbek, 2008; Çoruh, 2010; Çoruh, 2016). The damage level of the apple ermine moth is at significant levels Amasya, Ankara, Erzurum, Manisa and Van (Narmanlıoğlu & Çoruh, 2017).

Çoruh Valley is a very important transition zone that meets %97 of the fruit needs region. Naturally, there are many pests and diseases in the (Güçlü, Hayat, Özbek, Çalmaşur, & Pekel, 1998). *Yponomeuta malinellus* is also considerable pest of the region. In this case, the pest's parasitoids are very important. However, the number of studies on this subject is limited in our country (İren, 1960; Gençer & Doğanlar, 1996; Gençer, 2003; Narmanlıoğlu & Çoruh, 2017).

The aim of this study is to identify the new natural enemies of *Yponomeuta malinellus* and to investigate the activities of these beneficial species.

MATERIAL AND METHODS

Study area

Our investigations were carried out between 2019 and 2020. During this period 137 parasitoid specimens were made in the summers in the Çoruh Valley (Erzurum Province)

The Çoruh Valley has a special importance with its extraordinary fauna and flora, thanks to its climate. Due to its rich biodiversity, the region has been selected as one of the top 25 endangered ecological zones by Global Environment Fund (Aslantaş, Sönmez, & Demir, 2011).

The climate of the Çoruh Valley is particularly suitable for fruit production. Consequently, there are many types and numbers of fruit trees in valley (Karlıdağ & Eşitken, 2006) (Table 1).

Table 1. Fruit types and production quantities grown in İspir (Anonymous, 2022)

Fruit	Produce amount (da/kg)	Fruit	Produce amount (da/kg)
Pear	32	Cherry	27
Quince	25	Peach	18
Walnut	34	Sour cherry	25
Apple	174	Mulberry	63
Plum	24	Cranberry	13
Apricot	30	Grape	850

2022 Statistical Information System (Iva-Ibs) 3rd Term Finalized Data

İspir which is region settled in the Çoruh Valley, and its surroundings are located in the transition zone between Eastern Anatolia and the Eastern Black Sea Region. The Kaçkar Mountains in the north of the Middle Çoruh Valley and the Mescit Mountains in the south serve as a set that prevents the continental climate of Eastern Anatolia from directly affecting the region (Köse, 1991).

Sampling and collection method

A total of 560 *Yponomeuta malinellus* larvae were collected by hand from trees in study area (Fig. 1).

Figure 1. Map of study area.

Samples were collected from different apple orchards (Fig. 2) and different altitude (1220 m, 1229 m, 1239 m.) Samples were taken to represent 50% of the culturally cultivated apple orchards. The common apple trees were *Malus pumila* Mill. (Fig. 3). *Malus pumila* is a highly important commercial crop in the valley.

Figure 2. Pictures of study areas.

Figure 3. Infestation of *Yponomeuta malinellus* larvae on *Malus pumila*.

Each sample was placed in a box with clean leaves without any other harmful species apple leaves and covered with cheesecloth (Fig. 4). Cultured larvae were reared in a laboratory at ambient temperature to obtain parasitoids and were placed in groups of 10 in boxes (10 by 20 cm) for moth or parasitoid emergence. Periodically, withered leaves were replaced with fresh ones and checked every 1 or 2 days for 4 to 5 weeks. Emerging adults of parasitoids in the boxes were transferred to a killing jar.

Parasitoids identifications was verified by comparison with the preserved specimens in the Entomology Museum, Erzurum, Türkiye (EMET). The unidentified specimens were determined by specialists (Dr. Matthias Riedel and Dr. Saliha Çoruh).

Figure 4. Rearing boxes.

RESULTS

All the samples obtained from cultured larvae in the laboratory. These parasitoids are belonging to orders Hymenoptera. Among 137 samples, three parasitoid species *Itoplectis tunetana* (Schmiedeknecht, 1914), *Pimpla turionellae* (Linnaeus, 1758) and *Trieces facialis* (Thomson, 1887) belong to the family Ichneumonidae (Hymenoptera) (Table 2 and 3). *Trieces facialis* is reported for the first time for the Turkish fauna. In this study of the Çoruh Valley, the mean parasitism rate was %27.4. The present study increases the known Turkish Ichneumonidae to 1440 species.

Table 2. List of the parasitoids obtained from the *Yponomeuta malinellus* (2019-2020).

Parasitoid species	Number of individual parasitoids
Itoplectis tunetana (Schmiedeknecht, 1914)	48
Pimpla turionellae (Linnaeus, 1758)	67
Trieces facialis (Thomson, 1887),	22
Total	137

Table 3. Different data of parasitoids.

Names of Taxa	NH	Host food	ZR	Associated (plants)
Itoplectis tunetana	15	*Vaccinium uligino-sum,Vitis vinifera*	EP, E, WP	-
Pimpla turionellae	144	*Abies alba, A. sachalinensis, Beta vulgaris, Brassica olera-cea capitate, Euonymus euro-paeus, Fagus sylvatica, Hedya pruniana, Larix decidua, Malus domestica, Medicago sativa, Oryza sativa, Pinus contorta, P. res-inosa, P. sylvestris, Prunus avium, P. padus, Quercus pedunculata, Ribes nigrum, Ribes rubrum, Spina-cia oleracea, Vitis vinifera*	EP, E, NEAR, OCC, ORR, WP	*Adonis vernalis, Alnus glutinosa, Anethum graveolens, Anthriscus sylvestris, Carpinus betulus, Chae-rophyllum aromaticum, C. bul-bosum, Corylus avellana, Daucus carota, D. carota sativus, Epilobium angustifolium, Eu-phorbia nicaeen-sis, E. virgate, Fraxinus ex-celsior, Heracleum sphondylium, Junipe-rus communis, Malus domestica, Peucedanum oreoselinum, Picea excels, Prunus cerasif-era, Quercus robur, Sambucus nigra, Urtica dioica*
Trieces facialis	2	-	E, WP	-

Number Host (NH): Zoogeographical regions (ZR): E: Europe, EP: Eastern Palearctic, NEAR: Nearctic Region, OCC: Oceanic, WP: Western Palaarctic, ORR: Oriental.

DISCUSSION

This study was conducted in the same region in different localities between the years 2019-2020 to determine the parasitoids of harmful species in apple orchards.

A similar study to this study was carried out by the same authors in the same region before, and six parasitoid species were reared from *Yponomeuta malinellus*. Among these parasitoids, four species, *Diadegma armillatum* (Gravenhorst) *Trieces tricarinatus* (Holmgren) *Itoplectis tunetana* (Schmiedeckneckt) and *Itoplectis maculator* (Fabricius) (Ichneumonidae: Hymenoptera); one species, *Bessa parallela* (Meigen) (Tachinidae: Diptera) and one species, *Habrobracon concolorans* (Marshall) (Braconidae: Hymenoptera) Narmanlıoğlu & Çoruh, 2017).

Y. malinellus have been reported in previous studies in Turkey (İren, 1960; Junnikkala, 1960; Dijkerman, De Groot, & Herrebout, 1986; Kuhlmann et al., 1988; Gençer & Doğanlar, 1996; Gençer, 2003; Narmanlıoğlu & Çoruh, 2017).

This study was conducted in the same region in different lodges between the years 2015-2016. In 2019, the harmful damage increased again and the study was carried out again.

Similar results were observed in our previous study on parasitoid, assemblages of ectophagous Lepidoptera in apple orchard situated in Coruh Valley (Narmanlıoğlu & Çoruh, 2017).

Parasitoids of *Yponomeuta malinellus* have been reported in previous studies in Turkey (İren, 1960; Junnikkala, 1960; Dijkerman et al., 1986; Kuhlmann et al., 1988; Gençer & Doğanlar, 1996; Gençer, 2003; Narmanlıoğlu & Çoruh, 2017).

3 different parasitoid species were identified in this study:

Itoplectis tunetana is a species of Hymenoptera in the family ichneumon wasps. This is emerged from pupa of the Lepidoptera and has a solitary lifestyle. The host density are quite high (Yu, van Achterberg, & Horstmann, 2016). This has been olso obtained from *Y. evonymella* in Çoruh Valley on our previous study (Çoruh, 2005; Çoruh & Özbek, 2018; Narmanlıoğlu & Çoruh, 2017). Again, it is reported that this was obtained from different *Yponomeuta evonymella*, *Y. malinellus, Y. padellus*, *Y. rorellus* by many researchers (Özdemir & Kılınçer, 1990; Erol & Yaşar, 1996; Gençer, 2003; Özdemir & Özdemir, 2002; Çoruh & Özbek, 2008; Çoruh, 2016). *Itoplectis tunetana* has many different known hosts (*Acrolepiopsis assectella*, *Aleiodes gastritor*, *Anarsia lineatella*, *Ancylis sativa*, *Cacoecimorpha pronubana*, *Choreutis nemorana*, *Eupoecilia ambiguella*, *Lobesia botrana*, *Pandemis heparana*, *Parapandemis chondrillana*, *Plutella xylostella*, *Tebenna bjerkandrella*, *Tortrix viridana*, *Yponomeuta malinella*) worldwide (Constantineanu & Pisica, 1977; Talebi et al., 2005). It was obtained from %35.04 of the parasitized larvae (48 of 137).

Pimpla turionellae (Linnaeus, 1758) has been an important parasitic Hymenoptera species used in biological control for continuous management of pest (Gül, Özlük, & Özkorkmaz, 2013). This species emerged from larva/nymph, pupa, endoparasitoid, oviposit in larva/nymph, prepupa has a solitary lifestyle. The host density and the

number of associated plants are quite high (Yu et al., 2016). This species has obtained from 144 different hosts worldwide. It has also obtained from *Archips rosana, Archips spp., Cydia* sp., *Cydia pomonella, M. franconica, M. neustria, Yponomeuta cagnagella*, *Y. evonymella*, *Y. malinellus, Y. padellus*, *Y. rorellus* (İren, 1960; 1977; Soydanbay, 1978; Kansu & Uğur, 1984; Kansu, Kılınçer, Uğur, & Gürkan, 1986; Özdemir & Kılınçer, 1990; Öncüer, 1991; Kolarov, 1995, Erol & Yaşar, 1996; Özdemir & Özdemir, 2002; Çoruh & Özbek, 2008, Çoruh, 2016). *Pimpla turionellae* is used in biocontrol of *Choristoneura fumiferana*, *Euproctis chrysorrhoea*, *Lymantria dispar*, *Malacosoma disstria*, *Operophtera brumata*, *Orgyia pseudotsugata* and *Rhyacionia buoliana (*Yu et al., 2016). It was obtained from %48.91 of the parasitized larvae (67 of 137).

Piekarska-Boniecka, Rzańska-Wieczorek, Siatkowski, & Barczak (2022) found that *I. maculator* and *P. turionellae* in the apple ermine moth parasitoid complex.

Trieces facialis (Thomson, 1887) is a rare species. The is emerged from pupa, endoparasitoid, oviposit in larva/nymph and has a solitary lifestyle. The associated plant is *Oryza sativa* L. (Yu et al., 2016). It shows only in Finland, Sweden, Ukraine and Czechoslovakia distribution in the world. So far it has been only *Yponomeuta malinellus and Yponomeuta padella* in the world (Yu et al., 2016). It has been obtained from *Y. malinellus* our country first time with this study. Therewithal, *Trieces facialis* is a new record for the Turkish fauna. It caused %16.06 mortality of the specimens collected in this study (22 of 137).

The number of known Ichneumonidae species is currently 1439 (Doğru, 2022; İneciklioğlu, 2022). The present study increases the known Turkish Ichneumonidae to 1440 species.

When all these results are evaluated, it can be said that, the highest percentage of parasitism was recorded *Pimpla turionellae* in apple trees.

In this study, a new parasitoid of *Y. malinellus* was found the Çoruh Valley and the density of parasitoids-has been recorded.

We suggested that the list of parasitoids infesting larvae and pupae of *Y. malinellus* in Turkey is not completed yet. Studies have to be extended in the main fruit growing regions and Coruh Valley clarify parasitoid role as a mortality factor in various ecological conditions.

The study will help future laboratory and field studies to be carried out on this subject.

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An Egg parasitoid, *Mirufens* Girault (Hymenoptera: Trichogrammatidae) Reared from *Oxyrachis tarandus* Fab. Attacking on *Cassia fistula* L. (India)

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ABSTRACT

Cassia fistula L. is known as Golden shower or Amaltas has therapeutics importance in health care since ancient times. It shows a pivotal role in diseases prevention due to their valuable ingredients in different parts of plants. One of known hemipterous insect pest, attacking on this valuable tree is *Oxyrachis tarandus* Fab. (Hemiptera: Membracidae), its nymphs and adults feed gregariously on the sap of the shoot of host plant results in the stunting or death of the infested shoot. *Oxyrachis tarandus* population may be checked by some natural parasitoids. *Mirufens* Girault is one of important egg parasitoid recorded on this hemipterous insect pest, attacking on different host plants. In the present study, we are recorded egg parasitoid *M. afrangiata* from the eggs of *Oxyrachis tarandus*, infesting *Cassia fistula* plant for the first time. Re-described and illustrated two species of the genus *Mirufens*; *M. afrangiata* and *M. brevifuniculata* along with first male record of *M. brevifuniculata*. An updated key to the Indian species of *Mirufens* are also provided.

Keywords: Trichogrammatids, Biological control agent, Hemiptera, Amaltas.

Ikram, M., Yousuf, M., & Khan, S. (2023). An egg parasitoid, *Mirufens* Girault (Hymenoptera: Trichogrammatidae) reared from *Oxyrachis tarandus* Fab. attacking on *Cassia fistula* Linn. (India), *Journal of the Entomological Research Society*, 25(2), 305-312.

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INTRODUCTION

Cassia fistula L. (Fig. 1a) is commonly known as Amaltas/Indian laburnum/Golden shower has been widely used in different types of traditional medicines including Ayurveda, Unani and Chinese in the treatment and prevention of diseases. It shows a pivotal role in diseases prevention due to their valuable ingredients in different parts of plants such as stem, leaf, and flower. Some of the constituents (rhein, triterpenes, sugar, and potassium) show role as antimicrobial, anti-diabetic and antioxidant, and other types has therapeutic implications in cancer prevention (Rahmani, 2015). Important known hemipterous insect pest, attacking on this valuable tree is *Oxyrachis tarandus* (Hemiptera: Membracidae), widely distributed in India and adjacent countries. The nymphs and adults feed gregariously on the sap of the shoot of different host plants such as *Acacia catechu*, *A. nilotica*, *Albizia chinensis*, *A. lebbek*, *Cassia fistula*, *Dalbergia latifolia*, *Prosopis juliflora*, *P. cineraria*, *Santalum album*, *Tamarindus indica*. Females are laid her eggs on shoots in a V-shaped slit (Fig. 1b) and injury often results in the stunting or death of the infested shoot. Some natural parasitoids are also recorded, which may check the population and reduce the infestation of this insect pest. *Mirufens* Girault is important egg parasitoid belongs to the family Trichogrammatidae recorded on various hemipterous insects such as *Lapidosaphes ulmi* on *Betula* sp. (Erdos, 1956); *Oxyrachis tarandus* on *Acacia* sp. and *Prosopis juliflora* Viggiani & Hayat (1974).

Here, we have recorded egg parasitoid *M. afrangiata* from the eggs of *Oxyrachis tarandus*, infesting *Cassia fistula* plant for the first time. Re-described and illustrated two species of the genus *Mirufens*; *M. afrangiata* and *M. brevifuniculata* along with first male record of *M. brevifuniculata*. An updated key to the Indian species of *Mirufens* has been provided.

Figure 1. a) *Cassia fistula* plant (Amaltas), b) parasitized egg bunch of *Oxyrachis tarandus* on twig of *Cassia fistula*.

MATERIAL AND METHODS

Survey was conducted in forestry and agro-forestry areas of Haryana, and Uttar Pradesh (India) during June-July, 2018 and reared 14 specimens of egg parasitoid from egg bunches of *Oxyrachis tarandus* on twigs of *Prosopis juliflora* and *Cassia fistula*. Further, specimens were preserved in 70% alcohol. Following the normal process of dehydration, specimens were dissected in clove oil under stereoscopic microscope for studying the important morphological taxonomic characters; dissected body parts were kept in a drop of Euparol on slides and covered with cover slips. Only body lengths of specimens were measured in millimeters, all other measurements were taken from the divisions of a linear scale micrometer placed in the eye piece of a Nikon Digital Sight attached with Optiphot Microscope, at 10×, 20× and 40× (objective lens) for slide-mounted parts. Scales are placed on photographs of slide mounted parts and measurement was taken with the help of NIS-ELEMENT software in micrometer (µm).

Photographs of slide–mounted specimens were taken with digital camera "Nikon Digital Sight attached with Optiphot Microscope (Japan)" fitted over a compound microscope (Leica's Leitz Labor Lux S). All specimens were submitted to NFIC (National Forest Insect Collection), Forest Protection Division, Forest Research Institute, Dehradun, Uttarakhand (India).

The following abbreviations are used: OOL = Ocello-ocellar length; POL= Post-ocellar; C1 & C2= Club segments 1 & 2; FWW= Fore wing width; STV= Stigmal vein; MV= Marginal vein; PM= Pre marginal vein.

RESULTS

Key to Indian species of the genus *Mirufens* Girault based on females

Mirufens Girault includes 14 described (including four Indian species) valid species in the world (Noyes, 2022)

1. Fore wings with marginal fringe present; ovipositor arising from base of men…...2
- Fore wings with marginal fringe absent; ovipositor not arising from base of abdomen.....3
2. F1 and F2 wider than long, club less than 4× as long as wide; MV shorter than STV…….....….....……..….....*M. mangiferae*
- F1 distinctly longer than wide, about 1.5× as long as wide; club more than 4× as long as wide; MV as long as STV.....*M. longifuniculata*
3. Club slightly less than 3× as long as wide, F2 1.6× longer than F1, pedicel about 1.5× as long as wide (Fig. 2b); Fore wings with MV longer than STV (Fig. 2e); Mid lobe of mesoscutum about as long as wide, scutellum 1.5× as broad as long..... .....*M. afrangiata*
- Club more than 3× as long as wide; F1 and F2 about sub equal in length; pedicel more than1.5× as long as wide (Fig. 4b); fore wings with MV shorter than STV

(Fig. 4e); Mide lobe ofmesoscutum slightly wider than long, scutellum 1.2× as wide as long.....*M. brevifuniculata*

***Mirufens afrangiata* Viggiani & Hayat (Figs. 2-3)**

Mirufens (*Trachocera*) *afrangiata* Viggiani & Hayat, 1974: 145.

Mirufens albiscutellum Khan & Shafee, 1977: 32. Syn. by Hayat, 2008: 9.

Mirufens magniclavata Khan & Shafee, 1977: 32. Syn. by Hayat, 2008: 9.

Ufens afrangiata (Viggiani & Hayat): Yousuf & Shafee. 1988: 75.

Ufens albiscutellum (Khan &Shafee): Yousuf & Shafee. 1988: 77.

Ufens magniclavata (Khan & Shafee): Yousuf & Shafee. 1988: 80.

Re-description

Female: Body length, 0.59 mm Body dark except head, dorsal and ventral side of mesoscutum and scutellum with bright yellow; ocelli and eyes bright red. Antennae with club bright yellow with some dark infuscation on club segments. Fore wings hyaline except light infuscation beneath PMV; MV distinctly longer than STV. Ovipositor arising from 3rd gaster segment (Fig. 2a).

Head: Antennae (Fig. 2b) with scape about 4× as long as wide (81: 20); pedicel about 1.5× (44: 28) as long as wide; 2 indistinct ring segments present; 2 segmented of funicle (F1 & F2), F1 shorter than F2, F2 with long hairs as the hairs on club segments; club about 2.8× as long as wide (109: 38).

Mesosoma (Fig. 2c, d): Mid lobe of mesoscutum with (2+2) setae, about as long as wide (154: 152); scutellum each with 2+2 setae, about 1.5× as broad as long (126: 84); propodeum longer than dorsellum (30:19). Fore wings (Fig. 2e) hyaline except a light infuscated patch beneath PMV, slightly less than 2× as long as wide (524: 273); ratios of STV: MV: PMV: SMV, 56: 71: 66: 133, MV clearly longer than STV; disc with setae arranged in rows; RS1 with 5 setae; costal cell narrow; marginal fringe absent.

Metasoma: (Fig. 2f) Gaster longer than mesosoma; ovipositor arising from 3rd the level of gaster segment, slightly longer than hind tibia (97: 85).

Male: (Fig. 3a) Body length 0.58 mm, colour dark brown except fronto-vertex pale yellow. Antennae (Fig. 3b) with scape and pedicel same as female; funicle 2-segmented (F1 & F2), F1 slightly longer than F2 (37: 31); Club with 4 segments (C1, C2, C3 and C4), C3 longest (52) and C4 shortest segment. Fore wings (Fig. 3c) similar to female except marginal fringe present. Genitalia (Fig. 3d) with aedeagus clearly longer than apodemes, aedeagus and apodeme together slightly shorter than genital capsule.

Host: *Oxyrachis tarandus,* on *Acacia* sp.; *Oxyrachis tarandus* on *Prosopis juliflora; Parayasa elegantula; Oxyrachis tarandus* on *Mangifera indica; Oxyrachis tarandus* on *Cassia fistula* (New host plant record).

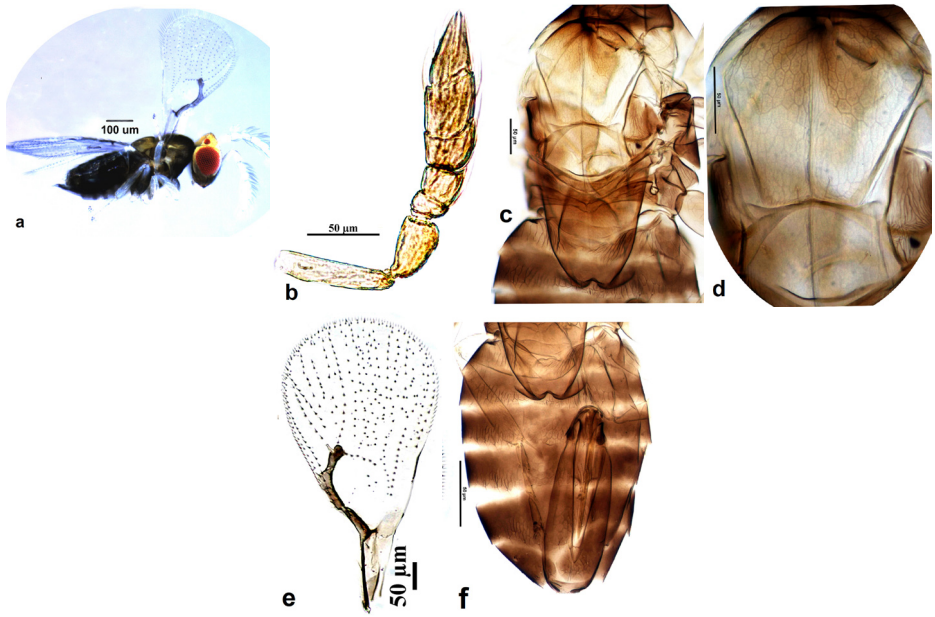

Figure 2. *Mirufens afrangiata* (Female). a) adult, b) antenna, c) mesosoma, d) midlobe of mesoscutum & scutellum (close view), e) fore wing, f) metasoma.

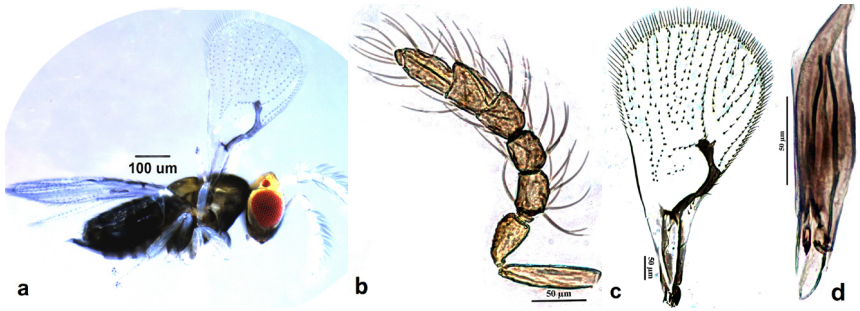

Figure 3. *Mirufens afrangiata* (Male). a) adult, b) antenna, c) fore wing, d) genitalia.

Material examined: INDIA: Uttar Pradesh: Saharanpur, Bahadarpur, 8♀♀ & 3♂♂ (on different slides), 28.06. 2018, M. Ikram; ex. Eggs of *Oxyrachis tarandus* on *Cassia fistula*.

Distribution: INDIA: Rajasthan, Tamilnadu, Uttar Pradesh, Punjab.

***Mirufens brevifuniculata* Khan & Shafee (Figs. 4-5)**

Mirufens brevifuniculata Khan & Shafee, 1977: 32.

Ufens brevifuniculata (Khan & Shafee): Yousuf & Shafee, 1988: 78.

Mirufens brevifuniculata Khan & Shafee: Hayat, 2008: 9.

Re-description

Female: Body (Fig. 4a) length, 0.64 mm. Body dark except head with fronto-vertex bright yellow; ocelli and eyes bright red. Antennae with club pale brown with some dark infuscation on apical segment of club. Fore wings hyaline except light infuscation beneath PMV; MV distinctly shorter than STV. Ovipositor arising from 3rd gaster segment.

Head: Antennae (Fig. 4b) with scape about 4× as long as wide (88: 22); pedicel 1.8× (44: 25) as long as wide; 2 indistinct ring segments present; 2 segments of funicle (F1 & F2), F1 and F2 sub-equal in length, F2 with short hairs; club about 3.4× as long as wide (121: 36), C2 about as long as C3.

Mesosoma: (Fig. 4e) mid lobe of mesoscutum with (2+2) setae, about 1.2× as long as wide (150: 130); scutellum with 2+2 setae, slightly broader than long (123: 101); propodeum longer than dorsellum (38: 22). Fore wings (Fig. 4c) hyaline except a light infuscated patch beneath PMV, slightly more than 2× as long as wide (542: 230); ratios of STV: MV: PMV: SMV, 66: 48: 64: 128, MV clearly shorter than STV (48: 66); disc with setae arranged in rows; RS1 with 3 setae; costal cell broad; marginal fringe absent.

Metasoma: Gaster longer than mesosoma; ovipositor (Fig. 4d) arising from 2nd level of gaster; 1.6× longer than hind tibia (249: 156).

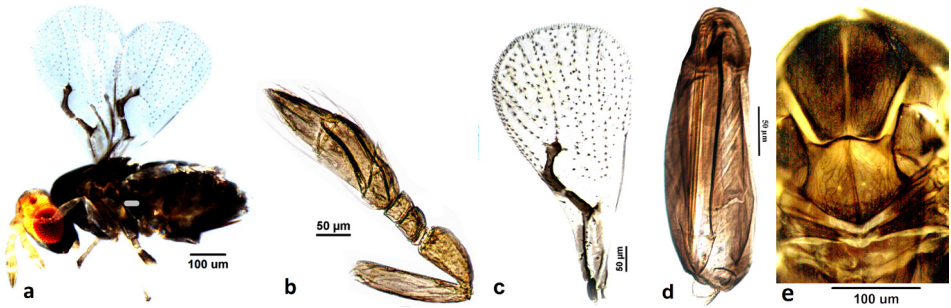

Figure 4. *Mirufens brevifuniculata* (Female). a) adult, b) antenna, c) fore wing, d) ovipositor, e) mesosoma

Male: (Fig. 5a) Body length shorter than female, 0.57 mm, colour dark brown except fronto-vertex and tibia, tarsal segments with pale yellow. Antennae (Fig. 5b) with scape and pedicel same as female; funicle 2-segmented (F1 & F2), F1 about as long as F2 (36: 35); Club with 4 segments (C1, C2, C3 and C4), C3 longest (56) and C4 shortest segment (9).Fore wings (Fig. 5c) similar to female except marginal fringe present. Genitalia (Fig. 5d) with aedeagus slightly shorter than apodemes, aedeagus and apodeme together slightly shorter than genital capsule (164: 141).

Host: *Oxyrachis tarandus,* on *Acacia* sp.; *Oxyrachis tarandus* on *Prosopis juliflora*

Material examined: INDIA, Haryana, Mahendragarh, Narnaul, 1♀ & 2♂♂ (on different slides), 14.07.2018, M. Ikram; ex. Eggs of *Oxyrachis tarandus* on *Prosopis juliflora*.

Distribution: INDIA: Rajasthan, Uttar Pradesh, Haryana (Present Record).

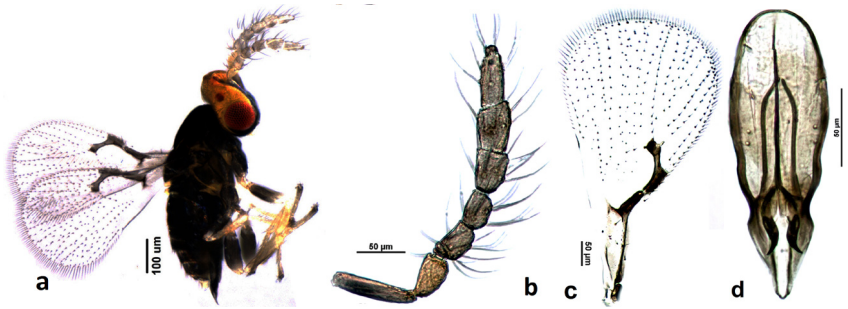

Figure 5. *Mirufens brevifuniculata* (Male). a) adult, b) antenna, c) fore wing, d) genitalia.

DISCUSSION

Oxyrachis tarandus (Hemiptera: Membracidae) is widely distributed in India and adjacent countries. Its nymphs and adults feed gregariously on the sap of the shoot of different host plants such as *Acacia catechu*, *A. nilotica*, *Albizia chinensis*, *A. lebbek*, *Cassia fistula*, *Prosopis juliflora* Yousuf & Gaur (1993). *Mirufens* is important egg parasitoid of insect pest of various hemipterous insects such as *Lapidosaphes ulmi* on *Betula* sp. (Erdos, 1956); *Oxyrachis tarandus* on *Acacia* sp. and *Prosopis juliflora*. *Oxyrachis tarandus* on *Mangifera indica*. So, there was no earlier record of parasitoid *Mirufens* on the eggs of *Oxyrachis tarandus* attacking on *Cassia fistula* but in present study, *Mirufens afrangiata* is being reported for the first time. Earlier worker poorly described and illustrated two species of *Mirufens*; *Mirufens afrangiata* and *Mirufens brevifuniculata* and there was no male record of *Mirufens brevifuniculata*. Thus, both species have been re-described in details with well illustration of different morphometric characters along with a revised key of the Indian species.

Cassia fistula has high medicinal value in different types of traditional medicines including Ayurveda, Unani and Chinese in the treatment and prevention of various diseases. So, It's important to protect this valuable tree from severe infestation of hemipterous insect pest, *Oxyrachis tarandus,* after developing mass multiplication technique of egg parasitoid, *Mirufens afrangiata* can be used as biological control agent for this pest. Re-description and updated key will be helpful in identification for future researchers.

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Research Article
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A New Species of *Oligoglena* Horváth, 1912 (Hemiptera: Cicadidae) from Mediterranean Region of Turkey

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ABSTRACT

Oligoglena sirintaylan sp. n. is defined from Saklıkent in Mediterranean Turkey, which are part of the Taurus Mountains. Taurus Mountains are known for their high diversity and endemism rate of both plants and animals. The new species has a characteristic morphology and is distinguished from all other species of the genus *Oligoglena* Horváth also by its behavioural character. It prefers the subalpine zone and lives in gramineous vegetation.

Keywords: Cicadettinae, *Oligoglena sirintaylan* sp n., morphology, acoustic signals

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INTRODUCTION

Anatolia is geologically teen massive. However, this area is one of the most active centres in all geological epoch. Due to intense volcanic and textural activity, different environmental conditions have led to the formation of high structures in dissimilar constitute (single mountains, mountain ranges) in the mountainous framework. In former western literature Anatolia is remarked to as Asia Minor by virtue of its high altitude (Turkmen, 2018).

Asia Minor divided four phytogeographical provinces of Turkey and these are: Mediterranean, Euxine, Mesopotamia, Irano-Anatolia. From these, Mediterranean includes the Mediterranean Taurus, Antitaurus, and Aegean Anatolia up to the Bursa provinces in the north (Çıplak, 2003).

The Taurus Mountains are lost of prominent for Anatolia biodiversity. In the course of history, ice-field refugia have become noteworthy centres of speciation and many endemic species have come in view in these areas. Many endemic species from the vertebrates, invertebrates, and plants also have been distributed in the Taurus Mountains (Çetintaş & Sözen, 2015). Taurus Mountains are located on the way of distribution Aegean plate species, via terrestrial corridors between Greece and Anatolia. These corridors created possibilities for faunal exchanges between the two sister plates, Anatolia, and Crete (Çıplak, 2004; Çıplak, Heller, & Willemse, 2010; Mol, 2020). This has been demonstrated for both some Cicada and Orthoptera species (Gogala & Trilar, 2014; Çıplak et al, 2010). This idea has been supported by making recent studies on *Oligoglena* Horváth, 1912 species (Hemiptera, Cicadidae) (Gogala,Tomi, & Drosopoulos, 2011; Mol, 2020).

Generally according to the results of the morphologically studies conducted at different time intervals, it is shown that 29 species belonging to Cicadidae family are distributions and nine of these are endemic in Turkey (Nast, 1972; Lodos & Kalkandelen, 1981; Duffels & Laan, 1985; Demir, 2007; Önder, Tezcan, Karsavuran, & Zeybekoğlu, 2011; Mol, 2020). Turkey, as well as the use of morphological characteristics of the species in taxonomic studies related to Cicadidae recently characterized, they also began to be used with acoustics characteristics (Zeybekoğlu et al., 2011; Mol, Zeybekoğlu, & Akyürek, 2013; Mol, 2017; 2020). Acoustics characteristics are used to appoint the taxonomic intercourse, in addition to that, it has been prospective to differentiate the buddy species and to elucidate the taxonomic status of vernacular populations with petty morphological differences by song patterns among the Cicada species, such as *Oligoglena* (Gogala, Puissant, & Trilar, 2017; Mol, 2020).

Previous studies reported that 12 species in the genus *Oligoglena* Horváth, 1912 distribution in the world (Dimitriev, 2017) and five of them have been recorded from Turkey: *O. parvula* (Fieber, 1876) (recorded from Amasya); *O. tibialis* (Panzer, 1798) (recorded from Ankara, İzmir, Hatay, and Adana); *O. sibilatrix* (Horváth, 1901) (recorded from İzmir, Mersin, and Kahramanmaraş), *O. turcica* (Schedl, 2001) (recorded from Gaziantep, Hatay, and Kahramanmaraş), and *O.gogalai* Mol, 2020 (recorded from Antalya) (Demir, 2019; Mol, 2020).

The main purposes of the present paper are to contribute to Anatolian biodiversity studies by determining a new species of the Turkish *Oligoglena* fauna by using the morphological descriptive characters and acoustics signals.

MATERIAL AND METHODS

Collecting of specimens

This study was carried out between 2015 and 2018 from Saklıkent of Antalya province which is located in the Mediterranean region of Turkey (Fig. 1). After having recorded the songs of *Oligoglena* males, they were collected with a sweeping net. The collected specimens were first put into alcohol and later stored as a dry material. Male genitalia were dissected and soaked into KOH at room temperature. Figures and measurements were obtained by using a digital camera (Leica DFC 295) and attached to stereo-microscope (Leica Z6 APO). Morphological studies were carried out using dry specimens after soaking them in alcohol and water. Terminology follows Moulds (2012) and Gogala et al., (2017). The specimens were diagnosed by comparing them with data provided in Gogala et al, 2008, 2009, 2017; Gogala,Tomi, & Drosopoulos, 2011; Gogala, Drosopoulos, & Trilar, 2012, 2017, Mol, 2020 and Boulard et al, 2022. The material is deposited in Aksaray, University Central Research Laboratory, Entomological Museum, (A. Mol collection), ASUBTAM (Aksaray/Turkey).

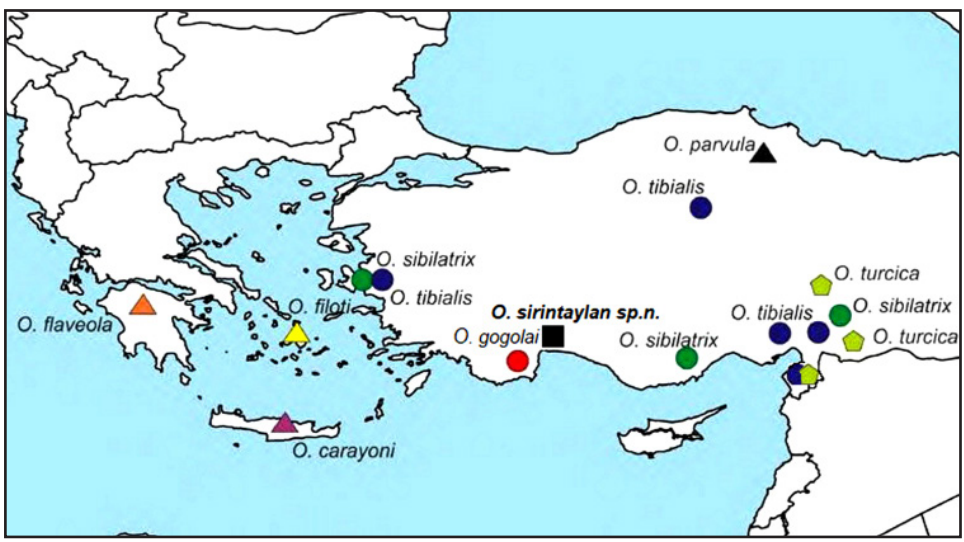

Figure 1. A map of Turkey with localities of all *Oligoglena* species found till now in Turkey including the new species, *O. sirintaylan* sp. n. (map revised from Mol 2020).

Song recording and analysis

Song recordings were made in the field using a Tascam DR-100mkII recorder with a Philips SBC ME 570 condenser microphone (frequency response from 50

to 20.000 Hz). The male songs were digitized at 48.000 samples per second and analyzed with custom-designed software (W. Schulze) developed in LabVIEW 7 (National Instruments, Austin, TX, USA) and Turbolab 4.0 (Stemmer AG). The ambient temperature at localities was during our recordings between 28-30°C. The Song terminology follows Trilar & Gogala (2010), and Gogala et al., (2017). The following terms were used: *Calling song*, song produced by a male; *phrase*, a first-order assemblage of echeme; echeme, the repeated unit of a phrase.

According to Gogala & Drosopoulos (2006) the song of *Oligoglena flaveola* (Brullé, 1832) comprises 4 tymbal clicks unit (inward movement of a tymbal producing a very soft clicks and the outward movement a loud one). Especially the duration of 4 double clicks unit is a very important bioacoustics characteristics for some *Oligoglena* species (Mol, 2020). In this study this characteristics were used to differentiation between new species and *O. gogalai* Mol, 2020.

RESULTS

A new *Oligoglena* species are described as a result of this study and now the species number has risen to six in Turkey.

Systematics

Family: Cicadidae Latrielle

Subfamily: Cicadettinae Buckton

Tribe: Cicadettini Buckton

Genus: *Oligoglena* Horváth, 1912

According to Gogala et al., (2017) and Dmitriev (2017), the genus *Oligoglena* can be characterised as it follows: Body length 11.9-12.7 mm, ratio length/width of fore wings 2.3-2.6 in males and 2.6-2.8 in females; fore wings rounded at apex; M and CuA meeting basal cell with their stems completely fused; hind wings with 5 apical cells; male terga II and III slightly enlarged and sternum VIII as long or slightly shorter than sternum VII; abdomen gradually narrowed caudad; claspers hooked anterolaterad; uncus small and duck-bill shaped; and the dorsal beak of pygofer well developed and basal lobe in ventral view showing inner tooh present.

Oligoglena sirintaylan sp. n.

Type material. Holotype (Male). Turkey, Antalya, Saklıkent, under the observatory, southwest slope, (N 36°50.03, E 030°18.38), 5.07.2015, 1887 m., Leg. A. Mol. Paratypes: 10♂, 5♀; 2018, 5♂, 2♀; Leg. A. Mol, D. Şirin, M. S. Taylan. The material is deposited of Aksaray University Central Research Laboratory, Entomological Museum (A. Mol collection), Aksaray, Turkey.

Description

Measurements: The body length from head to tip of abdomen is 12.8–14.4 mm in males and 15-17 mm in females; body length from the head to tip of tegmina is

18-20 mm in males and 17.2-19.5 mm in females. The tegmina length is 10.5-15.2 in males and 11-15 mm in females and maximum width tegmina is 4.5-6 in males and 4.5-6.2 mm in females (Table 1).

Male: Head black; middle of the ocellus and frons, supra-antennal plate and edge of postclypeus yellowish (Fig. 2c); mentum in basally yellowish, apically dark brownish in bottom and brownish yellow in laterally in the holotype and blackish in the some paratype. Rostrum blackish brown and extending to anterior margin of the nearly middle of third coxa; pronotum blackish, lateral angles of pronotal collar pronounced and yellowish, tip of the pronotal collar and ¾ of middle of the pronotum yellowish, some male paratype not yellowish; dark basal spot on pronotal collar diagonally semi-connected with both part of pronotum. Mesonotum black, with two yellow band in the middle of holotype, some paratype absent or H shaped pattern between submedian and lateral sigilla. Scutal depression black, cruciform elevation blackish in the holotype and brownish in the some paratype; lateral edge of it yellowish. Mesonotal posterior ridge near the wing groove yellowish, metanotum basilaterally and posteriorly yellowish (Fig. 2a)

Ventral side of thorax black with whitish yellow markings. All coxa brownish, with blackish markings (blackish in the some paratypes except edge); all trochanter blackish with yellow marking in the apically. Fore femora with three big and one small spines (Fig. 2d), tibiae blackish-brown, tarsus basally yellowish-black apically yellowish-brown. Pretarsal claws basally yellowish, apically brownish.

Abdominal sternite 1 on tip of the tymbals with whitish markings, terga 3–7 black with yellowish red borders. Tymbal membranes of the first abdominal segments (without tymbal covers) with four long and three short ribs in addition to the tymbal plate. Opercula kidney-shaped and whitish apically blackish basally, some paratype apically grayish, basally black, not overlapping, broadly rounded, with straight whitish spine (meracanthus) (Fig. 2b). Sternite I and II blackish. Abdominal sterna III through VI yellowish black with dark reddish margins. Apical half of sternum VII depression. Sternum VIII yellow, slightly shorter than sternum VII. Abdominal segments triangular in cross section, dorsally forming a rounded ridge. Half of Anepisternum 2, anepimeron 2, katepimeron and epimeral lobe blackish other parts whitish in the holotype and blackish white some paratype.

Tegmina and hind wings transparent, without markings, basally dark yellow, apically blackish. Tegmina not rounded at the apex, 2.42 times as long as wide, the paratype 2.38-2.60 times as long as wide. 8 apical cells on front wing, 5 on hind wing. Two paratype 9 apical cell in front wing and five paratype 6 apical cell in hind wing. Ulnar cell is I, 1.9 times longer than apical cell I, the paratype 1.4-1.95. Basal cell of the first wing transparent, basal membrane orange. Base of costal cell of hind wing orange. Veins basally yellowish, apically blackish, costal vein blackish laterally, upper yellowish, apically blackish; subcostal vein yellowish, basal cell vein dark yellowish; some paratypes all veins yellowish black (Fig. 2e). Head, pronotum, circle of eyes, behind of mesonotum, gena, lorum, coxa, trochanter, femur, anepisternum,

katepisternum, anepimeron covered with dense setae. Abdominal tergit and sternit including genitalia covered with sparse setae (Figs. 2b, 2f, and 2j).

The upper lobe and basal lobe of pygofer yellowish (Figs. 2h), remaining part blackish; some paratypes ventrally and basal half blackish, dorsally yellowish brown. Upper lobe of the pygofer positioned nearly middle of pygofer basal lobe of the pygofer with prominent spine (Fig. 2h). Aedeagus brownish (Fig. 2k). Uncus blackish brown, claspers short, opposite with basal lobe spine. Median lobe long; anal styles white with brownish marking longitudinally (Fig. 2i).

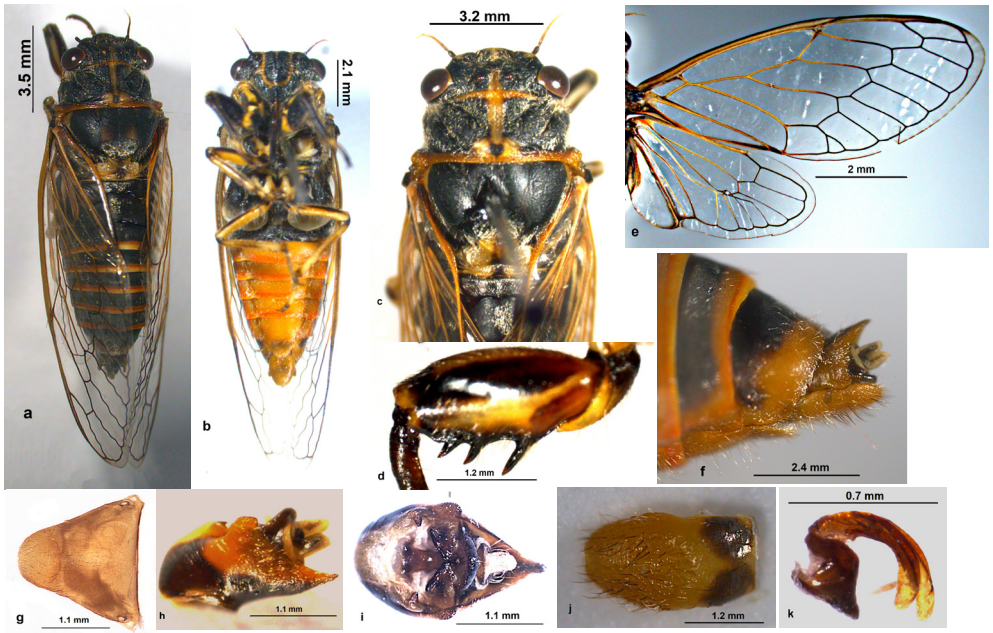

Figure 2. *O. sirintaylan* sp. n. male: a) male seen from above, b) body from below, c) head, pronotum mesonotum, d) fore femora, e) tegmina and wing, f) genitalia from lateral, g) sternite VII, h) pygofer from lateral, i) male genitalia from above, j) hypandrium, k) aedeagus.

Female: Generally differs from males in coloration. Mesonotum lighter than in males and with yellow H-shaped pattern between submedian and lateral sigilla (Figs. 3a and 3b). Tegmina 2.6-2.8 times as long as maximum width, Ulnar cell I is 2-2.5 times longer than apical cell I (Figs. 3e). Subgenital plate as in Fig. 3f, ovipositor 5.2 (4.2-6.2) mm and tip of it triangular with tubercules apically (Fig 3g). Tergite IX top and lower blackish and middle yellowish (Fig. 3f). Abdominal tergit and sternit including genitalia covered with long and dense setae (Fig. 3h).

Table 1. Morphological characteristics of *Oligoglena sirintaylan* sp. n. from Turkey. All measurements in mm.

Characteristics		Male		Female
	N	Average (range)	N	Average (range)
Tip of the cown to apical margin of the forewing	12	19 (18-20)	4	17.5 (17.2-18)
Body length	12	13.61 (12.8-14.4)	7	15.6 (14.8-16.5)
Length of crown	7	1.2 (0.91-1.6)	7	1.4 (1-1.8)
Min. distance betw. ocular sutures	7	1.90 (1.68-2.4)	7	1.96 (1.7-2.3)
Medial length of frons	7	0.51 (0.43-0.6)	7	0.5 (0.4-1.7)
Medial length of pronotum	7	1.65 (1.43-2)	7	1.74 (1.4-2.2)
Medial length of mesonotum	7	2.61 (1.95-3)	7	2.37 (1.9-2.8)
Length of right fore wing	7	12.2 (10.8-15.2	7	12.4 (11-15)
Length of ulnar cell 1	7	3.72 (3.2-5)		
Length of dorsal margin of left fore femur	6	2.2 (1.9-2.6)	7	2.21 (1.7-2.8)
Length of anterior margin of basal cell	7	1.2 (0.9-1.6)	7	1.23 (0.85-1.7)
VII. Sternit	6	1.92 (1.7-2)		-
Length of pygofer in lateral view	6	2.16 (1.9-2.3)		-
Aedeagus length	2	0.6-0.9		-
Hypandrium length (male) / Ovipositor length (female)	6	2.26 (2.0-2.5)	7	5.2 (4.2-6.2)

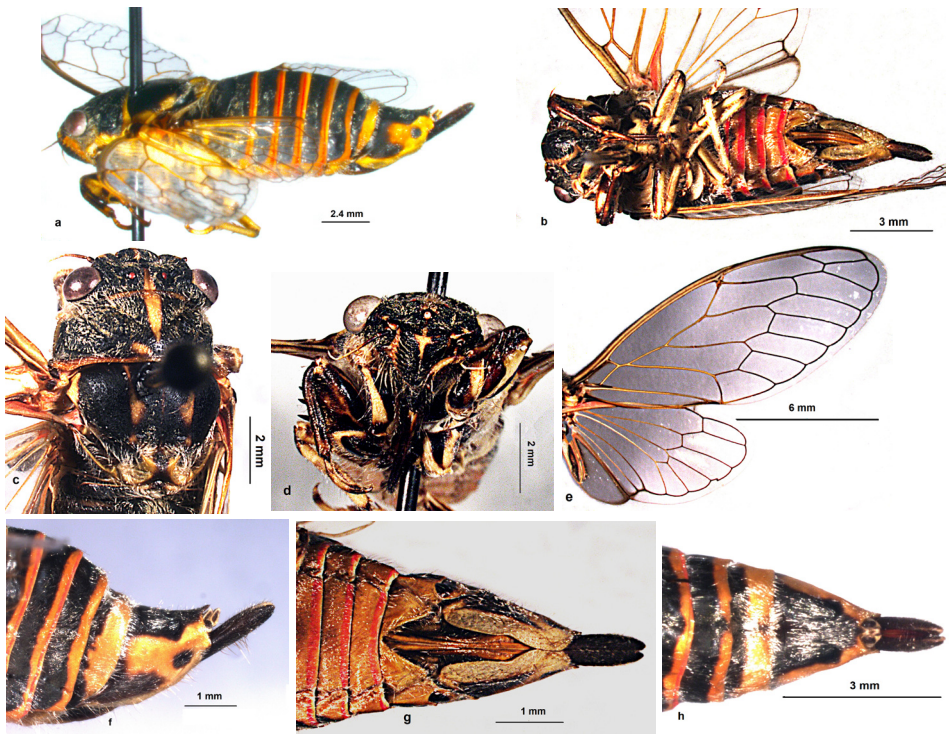

Figure 3. *O. sirintaylan sp. n.* female: a) body from lateral, b) body from bottom, c) head, pronotum and mesonotum, d) head from in front, e) tegmina and wing, f) genitalia from lateral, g) genitalia from bottom, h) genitalia from above.

Song pattern

At the locality, four different males were recorded song and analysed. Male calling song consist of two parts (monotonous and rhythmic part), together forming a complete song with usual duration of many minutes. The calling song consists of repetition of short echemes (S) of similar duration (monotonous-Phrase A) as a basic pattern (Fig. 4a). There are also further three phrases (rhythmic part-phrase B, C, and D) with more or less pattern of the longer (L) and shorter echemes (S). In the phrase B the echemes follow the pattern: LSSSLSSS…. (Fig. 4b), phrase C the echemes follow the pattern LSSLSS….(Fig. 4c), and in the phrase D the pattern is simpler LSLS….(Fig. 4d). Its calling songs consists of 4 tymbal clicks unit (Figs. 4e and 4f). Sometimes this pattern is also changing during a phrase from one to the other. The repetition rate of echemes in a phrase A is typically 9-12 echemes per second.

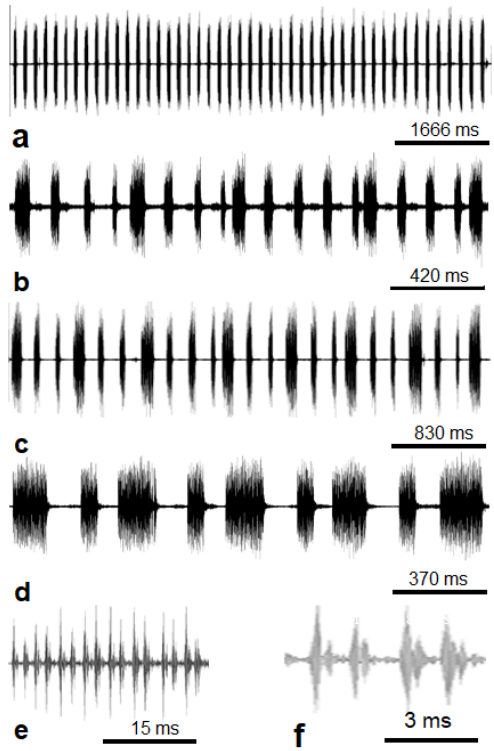

Figure 4. *O. sirintaylan* sp. n. male calling song. a) phrase A, b) phrase B, c) phrase C, d) phrase D, e) more 4 click units (scale= 15 ms), f) two 4 click units.

Element A duration ranges from 1.136-13.343 ms, with element B 342-548 (Table 2), element C 205-386, and element D 155-216 ms. Element A echemes duration 18-78, Element A interval duration between echemes 19-85, Element A echemes number/per seconds 9-12 (Table 3). Long echeme duration 41-84, 44-75, 41-77 ms in elements B, C, and D respectively (Table 4).

Table 2. Duration of elements A, B, C and D measured from male calling song of *O. sirintaylan* sp. n. population

Parameters	Element A durations	Element B durations	Element C durations	Element D durations
Range	1.136-13.343	336-452	205-386	155-216
M ± sd	(3089.2±2891	384±27	296.2±26.2	184.8±11.6
N	27)	5	96	76

Table 3. Song data belonging to element A measured from *O. sirintaylan* sp. n. population

Parameters	Element A Echemes duration	Element A Interval duration between echemes	Element A echeme number/ per second	Frequency spectrum (kHz)
Range	18-78	19-85	9-12	7.1-14.6
M ± sd	54.7±16.2	44.9±16.1	9.87±0.41	
N	854	854	67	

Table 4. Duration of long echemes belonging to elements B, C, and D measured from male calling song of *O. sirintaylan* sp. n. population

Parameters	Long echeme durations of element B	Long echeme durations of element C	Long echeme durations of element D	Totaly long echeme duration in Element B, C and D
Range	41-84	44-75	41-77	41-84
M ± sd	53.3±10	53.8±5.8	64.5±8.15	60.4±8.7
N	49	75	82	221

The duration of short echeme can last 12-36 ms, 12-73 ms, 15-37 ms in elements B, C, and D respectively. Interval between echemes range 20-89, 29-98, and 28-83 ms in elements B, C, and D respectively (Table 5 and 6).

Table 5. Duration of short echemes belonging to elements B, C and D measured from male calling song of *O. sirintaylan* sp. n.

Parameters	Short echeme durations of element B	Short echeme durations of element C	Short echeme durations of element D
Range	12-36	12-73	15-37
M ± sd	20.4±5	19.4±6.6	23.9±3.9
N	142	189	79

Table 6. Duration of interval between echemes belonging to elements B, C, and D and duration of 1 four click units measured from male calling song of *O. sirintaylan* sp. n. population

Parameters	Interval between echeme of element B	Interval between echeme of element C	Interval between echeme of element D	Duration 1 four click units
Range	20-89	29-98	28-83	3-4
M ± sd	60.7±14.2	58±14.5	43.8±10.2	3.06±0.25
N	193	397	154	302

The frequency spectrum of the song ranges from 7.1 to 14.6 kHz.

So far, five species of the genus *Oligoglena* have been reported from Turkey (Fig. 1), namely: *O. parvula* collected from Amasya, *O. tibialis* collected from Ankara, İzmir, Hatay, and Adana; *O. sibilatrix* collected from İzmir, Mersin and Kahramanmaraş; *O. turcica* collected from Gaziantep, Hatay, and Kahramanmaraş, *O.gogalai* collected from Antalya (Demir, 2019; Mol, 2020).

Etymology: Named after Dr. Deniz ŞİRİN (Namık Kemal Üniversity, Department of Biology, Tekirdağ/Turkey) and Dr. Mehmet Sait TAYLAN (Hakkari University, Health Services Vocational School Hakkari/Turkey) for their contributions to me recording calling songs and collecting new species materials in the study area.

Morphological diagnosis

O. sirintaylan sp. n. can be differentiated from *O. sibilatrix* using the following characters: (i) body length 13.61 (12.8-14.4) mm, (in *O. sibilatrix* 16 mm), (ii) tegmina basally dark yellow, apically blackish (in *O. sibilatrix* basally colourless, apically brownish), (iii) the ratio st VIII/VII is nearly 1.15-1.3, (in *O. sibilatrix* 2), (iv) the operculum rectangular (in *O. sibilatrix* squire), (v) under part of pygofer black (in *O. sibilatrix* pale), (vi) the habitat preferences (1887 m) subalpine zone and graminocolus vegetation (in *O. sibilatrix* habitat with forest zone, 623 m.) (Horváth, 1901).

Oligoglena sirintaylan sp. n. differs from *O. parvula* by the short pseudoparameres, which do not reach the middle of pygofer (in *O. parvula* at rest pseudoparameres long, pointed, reaching the middle of pygofer) and upper lobe of pygofer in the middle (in *O. parvula* uppler lobe of pygofer nearly located middle of apically quarter) (Schedl, 1999).

O. sirintaylan sp. n. can be differentiated from *O. tibialis* using the following characters: (i) the pseudoparameres are short, hardly visible, and not extending median lope of uncus (in *O tibialis* the pseudoparameres are long, divergend and much longer than median lobe of uncus), (ii) pronotal collar, its lateral part, anterior and posterior edge of pronotum yellow (in *O. tibialis* blackish), (iii) terga 3–7 black with yellowish red borders (in *O.tibialis* without), (iv) ulnar cell I is 1.5-1.95 times longer than apical cell I in male, 2-2.5 in female (in *O. tibialis* 1.25-1.42 in male, 0.85-1 in female), (v) hind wings with 5 apical cells in male (in *O. tibialis* 6), (vi) ovipositor length 4.2-6.2 mm (in *O. tibialis* 3.93-4.68), and (vii) pygofer inner teeth opposite to claspers (in *O. tibialis* not opposite) (Gogala & Drosopoulos 2006; Gogala et al., 2017; Mol, 2017).

O. sirintaylan sp. n. can be differentiated from *O. turcica* using the following characters: (i) five apical cells in hind wing (in *O. turcica* four), (ii) dorsal beak of pygofer long and acute (in *O. turcica*, short not acute), (iii) pygofer inner teeth under claspers (in *O. turcica* upper), (iv) dorsal beak reaching the tip of the anal styles (in *O. turcica* does not reach the tip of the anal styles), (v) meracanthus straight (in *O. turcica* curved inwards) (Schedl, 2001).

O. sirintaylan sp. n. can be differentiated from *O. gogalai* using the following characters: (i) sternite II black (in *O. gogalai* middle blackish), (ii) abdominal sterna III through VI yellowish black with dark reddish margins (in *O. gogalai* abdominal sterna III through VI yellowish with reddish-orange margins), (iii) half of anepisternum 2, anepimeron 2, katepimeron and epimeral lobe blackish other parts whitish (in *O. gogalai* anepisternum 2, anepimeron 2, katepimerron and epimeral lobe yellowish white), (iv) tergite IX lower blackish (in *O. gogalai* yellowish), (v) claspers short, opposite with basal lobe spine (in *O. gogalai* claspers long, surpass with basal lobe spine), (vi) ovipozitor 5.2 (4.2-6.2) mm (in *O. gogalai* 3.2 (3.1-3.3), (vii) meracanthus straight (in *O. gogalai* outwards) (Mol, 2020).

Based on morphology, the new species can be placed in the same group with *O. filoti* (Gogala & Trilar 2017), *O. flaveola* (Brullé, 1832), *O. carayoni* (Boulard, 1982) and *O. gogalai* (Trilar & Gogala 2010; Gogala et al., 2017; Mol 2020). Overall, it appears to be closest to *O. filoti* and *O. flaveola*. In particular, it displays very close affinities to *O. filoti* on the basis of dark body coloration, colour of pronotum, pseudoparamers and other parts of genitalia.

To distinguish *O. sirintaylan* sp. n. from *O. filoti*, the following characters are important: (i) lateral margin of pronotum, lateral part of the pronotal collar, and pronotal collar yellow (blackish in *O. filoti*), (ii) dark basal spot on pronotal collar diagonally semi-connected with both part of pronotum (completely connected with both part of pronotum in *O. filoti*), (iii) rostrum blackish brown and extending to anterior margin of the nearly middle of third coxa (reaching the posterior tips (distal end) of middle trochanters *O. filoti*), (iv) body with normal setae (dense setae in *O. filoti*), (v) sternum VII from middle to apical part with an elliptical depression absent in *O. filoti*), (vi) inner teeth of *pygofer* positioned lower, not opposite to claspers (opposite in *O. filoti*), (vii) mesonotum with yellow H-shaped pattern between submedian and lateral sigilla (absent in *O. filoti*) (Gogala et al., 2017).

To distinguish *O. sirintaylan* sp. n. from *O. flaveola*, the following characters are important: (i) dark basal spot on pronotal collar diagonally semi-connected with both part of pronotum (not connected in *O. flaveola*), (ii) terga 3–7 black with yellowish red borders (terga 3-7 yellow with lateral patches in both sexes *O. flaveola*), (iii) sternum I and II black (sterna I and II yellow, dark in middle in *O. flaveola*), (iv) the overall coloration of the male is black with yellow and dark reddish markings (overall coloration of the male is yellow in *O. flaveola*), (v) sternite I and II blackish (yellow in *O. flaveola*), (vi) the ratio st VIII/VII is 1.15-1.3 (0.86 in *O. flaveola*) (Gogala et al., 2017).

To distinguish *O. sirintaylan* sp. n. from *O. carayoni* (Boulard) the following characters are important: (i) the overall coloration of the male is black with yellow and dark reddish markings (nearly black in *O. carayoni*), (ii) lateral angles of pronotal collar yellowish (black in *O. carayoni*), (iii) basal membran of tegmina orange (dark red in *O. carayoni*), (iv) abdominal terga 2 through 7 black with yellowish red borders (black with dark red borders in *O. carayoni*), (v) *O. carayoni* is thought to be endemic to Crete (Trilar & Gogala, 2010; Gogala et al., 2017).

Acoustic diagnosis

The new species has some differences from *O. filoti* in terms of acoustic characteristics, which include the following: (i) the duration of echemes in phrase A is 54.7±16.2, (in *O. filoti* 25.5±4.3), the duration of interval between echemes in phrase A is 44.9±16.1, (in *O. filoti* 98.7±6.5), iii) phrase B duration of short echeme is 20.4±5 (in *O. filoti* 7.7±4.5 ms), (iv) the frequency spectrum is in the range of 7.1-14.6 kHz (in *O. filoti* 10.8-17.8 kHz), and (v) the number of short echemes between long echemes is 1-3 (in *O. filoti* stable 2) (Gogala et al., 2017).

The new species has the following differences from *O. flaveola* in terms of acoustic characteristics: (i) duration of 4 tymbal clicks is 3.06±0.25 ms (in *O. flaveola* 8±1), (ii)

short echemes in the basic phrase A are often composed more than 4 of such basic 4-clicks units (in *O. flaveola* it composed from 3 and sometimes 2), song consist of SSSS/LSSSL/LSSL/LSLS (in *O. flaveola* SSS/LSSL/ LSLS), the frequency spectrum ranges between 7.1-14.6 kHz (in *O. flaveola* 13.5-16 kHz) (Gogala et al., 2017).

The new species has some differences from *O. carayoni* in terms of acoustic characteristics and some of these are: i) song structure SSSS/LSSSL/LSSL/LSLS (in *O. carayoni* SSS/LSSL/LLL), ii) the duration of interval between echemes in phrase A is 44.9±16 (in *O. carayoni* 91.6±14.6), iii) the duration of echemes in phrase A is 18-78 (54.7±16.2), (in *O. carayoni* 14.0-37.9), iv) rhythmic part consists of series with 1 to 3 echemes followed by one longer echeme (in *O. carayoni* 2 to 4 very short echemes), v) duration of 4 tymbal clicks is 3.06±0.25 ms (in *O. carayoni* 8±0.9), the frequency spectrum ranges from 7.1-14.6 kHz (in *O. carayoni* carrier frequency maximum is between 10.8 and 14.7) (Trilar, & Gogala, 2010; Gogala et al., 2017; Boulard et al., 2022).

The new species has some differences from *O. gogalai* in terms of acoustic characteristics and some of these are: i) it has low amplitude song (in *O. gogalai* high amplitude), ii) the duration of element D is 184.8 ±11.6 (in *O. gogalai* 309.2±20.4), iii) the duration of echemes in phrase A is 54.7±16.2 (in *O. gogalai* 24.2±9), iv) duration of 4 tymbal clicks is 3.06±0.25 (in *O. gogalai* 5±1), the duration of interval between echemes in phrase D is 43.8±10.2 (in *O. gogalai* 120±52.6).

Ecology: The new species sitting on the grass and herbaceous plants close to the ground and perennial plant (Fig. 5). At the locality, no other songs of Cicadidae species were recorded.

CONCLUSIONS AND DISCUSSION

According to Mol (2020) and as a result of this study, the species number has risen to six in Turkey and these are; *O. parvula*; *O. tibialis*; *O. sibilatrix*, *O. turcica, O. gogalai* and *O. sirintaylan* sp. n. Of these six species, the last three are endemic for Anatolia. All three endemic species are distributed in the Taurus Mountains, which proves how important the Taurus Mountains are in terms of plant and animal diversity.

Based on morphology and bioacoustics characteristics, the new species can be placed in the same group with *O. filoti*, *O. carayoni*, *O. flaveola*, and *O. gogalai* (Trilar & Gogala 2010, Gogala et al., 2017, Mol, 2020). First tree species endemic for Eagean island and *O. flaveola* recorded from in the Peloponnesse, continental Greece (Gogala et al., 2017). Faunistics resemblance between the Turkish mainland and Greece were reported for some cicada species: *Lyristes gemellus* Boulard, 1988 and *Pagiphora aschei* Kartal,1978 (Trilar & Gogala, 2012; Simoes & Quartau, 2013).

Studies conducted in recent years (Mol, 2020, and this study) show us that there is still a lot of work to be done for Turkey's *Oligoglena* species.

Figure 5. *O. sirintaylan* sp. n. type locality. Photograph: Dr. Deniz ŞİRİN.

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Natural Enemies of *Planococcus vovae* (Nasonov) (Hemiptera: Coccoidea: Pseudococidae), the Main Pest of Lawson's Cypress Trees, in Northeast Iran

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ABSTRACT

There is increasing interest in using natural enemies to control pests in urban green spaces, but this is often hampered by a lack of information on enemies of particular pest groups. Here we provide an assessment for the first time of seasonal population of coccid species and its natural enemies on *Chamaecyparis lawsoniana* (A. Murray) Parl, 1864. The main coccid pest, *Planococcus vovae* (Nasonov, 1909), is attacked by parasitoids and predators. According to the gathered data, the peak population of both coccids and their natural enemies was observed in spring. The most common species in natural enemies was, *Scymnus syriacus* Marseul,1868. Biodiversity indexes of the natural enemies community in the selected stations demonstrated the highest and lowest amounts for both Shanon winner and Margalef index in S3 and S1 before and after for the Simpson index in S1 and S4,5. This study suggests that the natural enemies of coccids on conifer trees may be more complex and diverse than what was observed in the present study.

Keywords: Conifers, Cupressaceae, Predator, Parasitoid, Pest.

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INTRODUCTION

Compared with other metropolises, Mashhad has been particularly affected by rapid urbanization and associated socio-ecological dynamics. In the last census in 2016, the population of 3,312,090 was recorded for Mashhad. Also, more than 27,000,000 visitors annually are incoming Mashhad from all over the world (Ghaderpoori et al, 2016). It has been suggested that urban greening confers a wide variety of socio-ecological benefits to residents and urban environment, a fact that is increasingly appreciated (Lo et al, 2017). Stresses, such as drought, high temperatures and even the factors arising from anthropogenic disturbance, may expose trees to pest attacks, resulting in the pests establishing and increasing in density (Paap et al, 2017). Conifers are one of the most important trees in the most regions of urban green spaces (Heidari et al, 2020).

The coccid species, *Planococcus vovae* (Nasonov, 1909), a secondary insect pest of Cupressaceae species (Japoshvili & Karasa, 2002), is found on most of Lawson cypress trees *Chamaecyparis lawsoniana* (A.Murray bis) Parl., in Iran (Talebi et al, 2008). *P. vovae* presence occurs frequently, but little insecticide is used for its control because it rarely reaches economically damaging levels as well as using insecticide side effects on the environment in urban green spaces are more than their benefit (Choumert et al, 2008). However, short-lived outbreaks in some cases cause considerable damages, which is need led to various control measures (Vesey-Fitzgerald, 1953).

Natural enemies' abundance is a major factor that affects the population dynamics of herbivorous insects (Heidari Latibari et al, 2021, Diaxon and Kindmann, 1990). *P. vovae* uses specific host plant species of family Cupressaceae, with the no previous report of this species in northeast of Iran (Moghaddam, 2013). Although few studies have been done on *P. vovae* in Iran (Moghaddam & Nematian, 2020), the limited evidence suggests that natural enemies play a major role in control of this pest (Tamoli Torfi et al, 2020). Here, we report results of the first enclosure field survey on *P. vovae* and its natural enemies in northeast of Iran.

MATERIAL AND METHODS

Study sites and sampling design

Sampling was carried out in the planted Lawson's cypress planted in the urban green spaces of Mashhad, Khorasan Razavi province, Iran (36°15'N, 59°37'E, 985 m a.s.l). The maps both were elaborated by Google Earth (Fig. 1). Sampling was conducted weekly from March 2020 to February 2021 in the four seasons of six preselected sites. Fifteen Lawson cypress trees approximately with 2m height were selected. on the basis of previous samplings that revealed severe infestations by coccids. The sampling unit was 20cm terminal branches haphazardly selected from two vertical divisions of the canopy. Each branch was separately put inside a plastic bag and then cut and moved to the laboratory for more studies (Heidari Latibari et al, 2016).

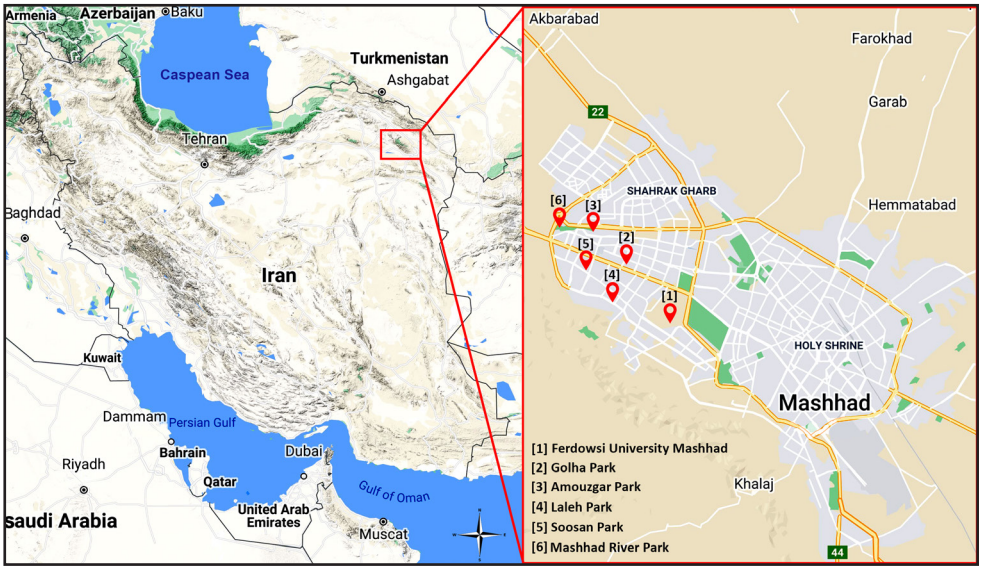

Figure 1. Location of the study area and the selected stations in Mashhad city (Razavi Khorasan province, Iran).

Collection, preparation, and identification

Infested branches were immersed in water and kept individually inside cylindrical cages. Upperparts of cages were covered with mesh to allow sufficient ventilation. Also, some branches were put inside the Petri dishes. Each cage and Petri dishes were properly labeled with the collection date and the serial number of the contained branches. The branches and Petri dishes were transferred in a growth chamber set at 25°C, 56% (RH), and a 16:8 (L:D) h photoperiod, till emerging the adults of parasitoids as well as coccinellids. Adult newly emerged insects were stored in 75% ethanol and were labeled using the new pinning block after preparation and mounting (Ghafouri Moghaddam et al, 2017). All collected coccid species were preserved into 75% ethanol. Some specimens, were slide-mounted on Canada balsam for more details. Coccids and their natural enemies were identified using available keys (Hayat, 1983; Nedved, 2015). Expert taxonomists reidentified and confirmed adult specimens to the species level.

Statistical analysis

Coccids and their natural enemies in the samples were separated and counted. The mean number of coccids per branch and the total number of coccids were calculated. Frequencies of coccids and their natural enemies, and biodiversity indexes; Margalef, Shanon winner, and Simpson were estimated for natural enemies. Data were statistically analyzed in SPSS V 22.

RESULTS

Collected species

The coccid species, *P. vovae* and eleven species of natural enemies have been collected. A total of 2495 pine-feeding coccid species were found on Lawson cypress from six station in urban green spaces of Mashhad. It is first report of *P. vovae* from Razavi Khorasan province. The coccids population on *C. lawsoniana* reached its peak abundance at the end-March till the mid-May in station number 4 and also there was a minor peak between mid-November and end-December in station number 3. During subsequent weeks a gradual decrease in the coccid density was observed, but it did not reach zero (Fig. 2).

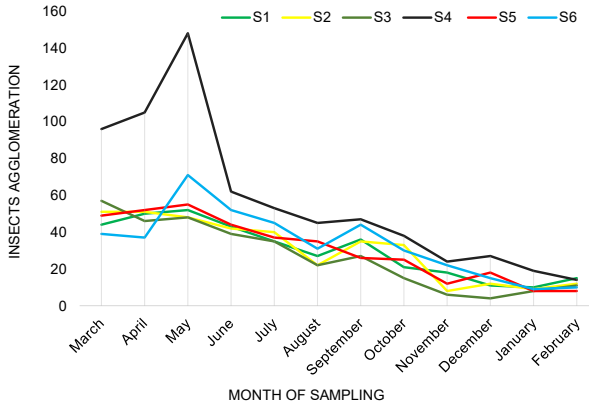

Figure 2. Population dynamics of Planococcus vovae during March 2020-February 2021 at stations 1 to 6 (S1-S6).

Natural enemies

Over the period 2020–2021, we collected and identified 337 individuals of coccid natural enemies belonging to three order four family and eight species (Table 1). According to their densities on Lawson cypress trees, *Scymnus syriacus* Marsuel, 1868 with the highest occurrence frequency among collected specimens, were considered as the dominant species (bolded in table 1). In contrast, *Scymnus nubilus* Mulsant, 1850 and *Coccinella septempunctata* (Linnaeus, 1758) were much less common. The coccinellid species *Chilocorus bipustulatus* (Linnaeus, 1758) pre-dominated. *Chrysoperla carnea* (Stephens, 1836) recorded as a predator as well. Only two species of parasitoids wasps; *Aphytis mytilaspidis* Le Baron, 1870 and *Aphycus secundus* (Mercet, 1925) have been observed from parasitized coccids (Table 1). The low numbers of natural enemies were present in winter but density peaked between early spring and also kept approximately till the start of fall. A comparison of biodiversity indexes showed that S3 and S1 stations had the highest and lowest Shannon Wiener values between selected stations (Fig 3). For Margalef, the highest and lowest amounts

were observed in S3 and S1 (Fig 4) while for Simpson, the highest and lowest values were also observed in S1 and S4,5 (Fig 5).

Table 1. The frequency of natural enemies at selected stations during March 2020 and February 2021.

Family	Species	Station	Spring	Summer	Fall	Winter
Coccinellidae	*Chilocorus bipustulatus* *Coccinella septempunctata* *Nephus bipunctatus* *Scymnus nubilus* *Scymnus syriacus*	1, 3, 4, 6, 5 1, 2, 3, 5, 6 3, 4, 5 2, 3, 6 1, 2, 3, 4, 5, 6	21 10 27 13 57	13 5 10 1 41	7 10 1 5 20	5 7 3 0 8
Chrysopidae	*Chryspoerla carnea*	3, 4, 5	20	18	5	6
Aphelinidae	*Aphytis mytilaspidis*	3	1	2	0	0
Encyrtidae	*Aphycus secundus*	2	0	1	0	0

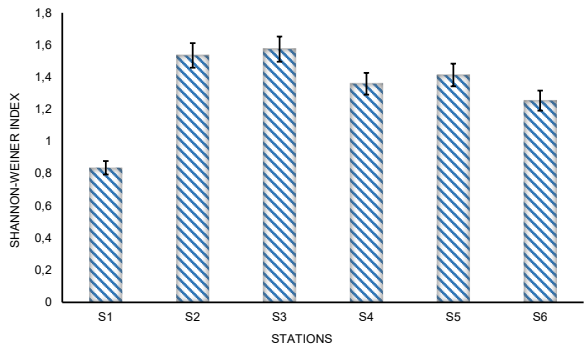

Figure 3. Shannon-Wiener diversity index of natural enemies at each sampling station.

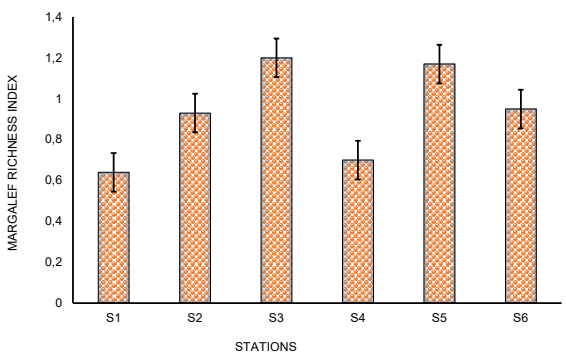

Figure 4. Margalef diversity index of natural enemies at each sampling station.

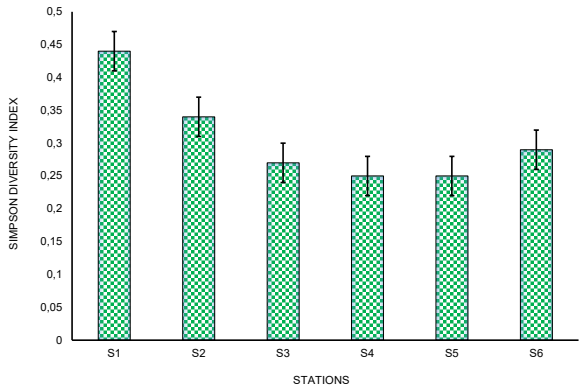

Figure 5. Simpson diversity index of natural enemies at each sampling station.

DISCUSSION

The abundance and diversity of insects are both affected by urbanization. Determining how pests and natural enemies are affected by urban environments will help inform how we design more resilient urban landscapes that protect tree health and functioning (Parsons & Frank, 2019). Here, we selected six sampling sites with Lawsonia trees that were infested by colonies of coccids with different amounts, to examine how the urban environment affects the recruitment of coccids and their natural enemies structure, which in turn will feedback and affect hence plant traits.

Based on our collected date, *P. vovae* is likely to face attacks from a community of natural enemies (Stathas et al, 2021). Despite the lack of conclusive evidence of their level of effectiveness, they could potentially contribute to the control of this species (Perez-Alvarez et al, 2019). This study has revealed that two parasitoid and six predator species are active as attacking *P. vovae* in the urban green space of Mashhad. The wide distribution and large number of *S. syriacus* collected suggests that this species is the most abundant predator of *P. vovae* in the selected regions. The importance of predators as natural enemies of *P. vovae* was alluded to in the literature from last century (Flanders, 1943; Beardsley, 1955; Pimentel, 1963). We observed *S. syriacus* as the abundant species in natural enemy's complex of scales, however *Nephus bipunctatus* (Kugelann, 1794), *C. bipustulatus* and *C. carnea* were more common in previous literatures (Lotfalizadeh & Ahmadi, 2001; Talebi et al, 2008). This appears to be the first publication that refers to *C. septempunctata, S. syriacus*, *A. secundus* and *A. mytilaspidis* as natural enemies of *P. vovae* in Iran.

In the future studies related to the biological control of populations of *the P. vovae*, recommended a special importance given to *S. syriacus* as it seems this species have potential to control population of this pest. This study enlightened to have a holistic approach for the better management of economically important coccids by using

potential natural enemies (particularly coccinellids) to increase the health quality of conifers in urban green spaces.

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Research Article
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Scanning Electron Microscope Studies on Ornamentation of Egg Chorion of *Capissa vagesa* (Moore, 1859) (Erebidae) and *Trabala vishnou* (Lefèbvre, 1827) (Lasiocampidae) (Ditrysia: Lepidoptera) from India

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ABSTRACT

The eggs of *Capissa vagesa* (Moore, 1859) and *Trabala vishnou* (Lefèbvre, 1827) from the families Erebidae and Lasiocampidae were examined and characterized under scanning electron microscope. Significant morphological traits on the eggshells of both analyzed species are presented in current study. Descriptions and comparative morphological assessments for both species of these moths are provided, in addition to the structural complexity of the eggs uncovered throughout the course of this study. This study showed that ultrastructural egg chorion features investigated in the current study i.e., shape of Micropylar rosette, polygonal cells, number of micropyles and aeropyles have high taxonomic significance at specific and generic levels, and these sort of investigations must be expanded to improve and elevate the morphological depiction at levels of earlier life stages in various moth families.

Key words: Aeropyles, micropylar rosette, micropylar pit, micropyles, polygonal cells.

Kaleka, A.S., Jallundhara, S., & Kapoor, Y. (2023). Scanning electron microscope studies on ornamentation of egg chorion of *Capissa vagesa* (Moore, 1859) (Erebidae) and *Trabala vishnou* (Lefèbvre, 1827) (Lasiocampidae: Ditrysia: Lepidoptera) from India. *Journal of the Entomological Research Society*, 25(2), 337-349.

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INTRODUCTION

Invertebrates such as insects, spiders, mollusks, and crustaceans have long used eggs as a mechanism of reproduction. Lepidopterans, like most other insects, are oviparous, or “egg-bearers” (Gullan & Cranston, 2004). Many species deposit their eggs individually, in widely dispersed clumps, or in masses, while others lay them in masses covered with a hardened fluid from the female’s abdominal glands (Holland, 1898, 1903).

In Lepidoptera, the eggs are usually oval, spherical, dome-like, disc-like, conical, cuboid or irregular in shapes (Peterson, 1964). The chorion may have a simple and basic structure, or may have a reticulate patterned surface with divisions and transverse or longitudinal ridges along with other parts of chorion i.e., polygonal cells, micropylar rosette and micropyles. The chorion is the outer protective layer of the egg and secreted by the ovarian follicle cells along with the surface sculpturing (polygonal cells) of the chorion which usually reflects the outline or imprints of these cells. The egg shell typically has tiny minute grooves or holes (airspaces, air-pores, or aeropyles) that can only be seen using an electron microscope’s high magnification and resolution capabilities. These air-pores allow for the exchange of oxygen (O_2) and carbon dioxide (CO_2) between the environment and embryo with very little water loss. The egg’s anterior pole is slightly flattened, resulting in a tiny medial chamber with a minute hole known as a micropyle (Evans, 1932).

The chorion is secreted around the whole egg while it is inside the ovary, so it becomes obligatory to have amenity for permitting the upcoming entry of sperm through it. The small openings which allow the sperm to enter and fertilize the ovum are termed as micropyles. These small funnel shaped pores which run and pass right through the chorion are usually located near the anterior pole of the egg (Salked, 1975).

The micropylar zone is found on the upper anterior section of globular, conical, or cylindrical eggs, as well as on the outside perimeter or rim of flattened or lenticular eggs (Holland, 1898, 1903). This area is protected by a unique arrangement known as micropylar rosette. The number of micropyles ranges from 1 to 20 in Lepidoptera (Hinton, 1981).

There are several methodologies for analyzing taxonomic characteristics, such as morpho-taxonomy, molecular taxonomy, behavioral taxonomy, and ecological taxonomy, among others. The current effort is precise advantage in terms of improving and elevating the morphological characteristics of various moths. These scanning electron microscopic studies will build up the morphological representation of the external surface characteristics of the eggshell i.e., chorion and reveal both traditional and mutable features, thus serve in learning various facets of biological diversity. Such studies will surely provide valuable tools to disclose the eggshell sculpturing in various lepidopteran families at species and generic levels in order to segregate them at much earlier stages of their life histories which can prove beneficial in case of pests as earlier identification of pests through eggs on economically important plants will help scientists and farmers in building a proper and more suitable pest management program.

MATERIALS AND METHODS

Collection and identification The tours were conducted in Sainj and Shalwad localities of the district Kullu in Himachal Pradesh to collect the adults as well as eggs of moths in September 2020. The adult specimens were stretched, preserved and identified.

Material examined

Capissa vagesa, Himachal Pradesh: Sainj, 2 eggs and *Trabala vishnou,* Himachal Pradesh: Shalwad, 3 eggs

Taxonomy and distribution

Genus: *Capissa* Moore, 1878

Capissa Moore, 1878; *Proc. zool. Soc. Lond.,* 1878: 19; Moore, 1882, *Lepid. Ceylon,* 2 (1): 56, 134; Singh, Singh & Joshi, 2014, *Rec. Zool. Survey India. Occ. Pap.,* 367: 27.

Type species. *Lithosia vagesa* Moore, 1859

Distribution. India: Assam, Kashmir, Sikkim, North-West Himalayas; Nepal, Myanmar.

Remarks. Genus *Capissa* Moore, 1878 is a monotypic tiger moth genus of the family Erebidae. It was formerly considered a synonym of the genus *Eilema* Hübner, 1819. This genus was firstly reported by Frederic Moore in 1878. The adult form usually found from North West Himalayas and Nepal has the legs with black bands at joints and the eastern form of this moth has wholly black legs.

Species: *Capissa vagesa* (Moore, 1859)

Lithosia vagesa Moore, 1859; in Horsfield & Moore, *Cat. Lep. Ins. Mus. Nat. East India Hous*e 2: 304.

Capissa vagesa; Kirti, Singh & Joshi, 2014, Ann. Zool. 64 (1): 46; Singh, Singh & Joshi, 2014, Rec. Zool. Survey India. Occ. Pap 367: 27, 134.

Ilema vagesa; Hampson, 1900, Cat. Lep. Phalaenae Br. Mus. 2: 144, f. 91.

Eilema vagesa; Kishida, 1993, Tinea 13: 37, pl. 40, f. 15.

Eilema vegesa; Kishida, 1994, Tinea 14: 69.

Type locality. Sikkim (India)

Distribution. The species is distributed throughout Indian Himalayas, Nepal and Myanmar.

Remarks. The monotypic status of this genus has been updated recently as Kirti et al (2014) described a new species, *Capissa alba* sp. nov., from Jammu and Kashmir region of Indian Himalayas. During the present study, the ultrastructure details of the eggs of *Capissa vagesa* (Moore, 1859) have been studied for the first time.

Genus: *Trabala Walker, 1856*

Walker, 1856, *List Spec. Lepid. Insects Colln. Br. Mus.,* 7: 1785; Moore, 1883, *Lepid. Ceylon,* 2: 146; Hampson, 1892, *Moths India,* 1: 421; Holloway, 1987, *Moths Borneo,* 3: 49; Zolotuhin, Treadaway, & Witt, 1997, *Lasiocampidae Philippines,* 17: 139; Zolotuhin and Witt, 2000, *Lasiocampidae Vietnam,* 3(11): 48; Zolotuhin & Pinratana, 2005, *Lasiocampidae Thailand,* 4: 41; Youqiao and Chunsheng, 2006, *Fauna Sinica,* 47: 333-334; Sujata, Kaleka, & Singh, 2019, *Ann. Entomol.*, 37(1): 19-27.

Amydona Walker, 1855, *List Spec. Lepid. Insects Colln. Br. Mus.,* 6: 1387.

Type species. *Amydona prasina* Walker, 1855

Distribution. Oriental and Palearctic regions.

Remarks. This genus was established as an objective replacement name for *Amydona* Walker and the type species of this genus was designated by Moore in 1883. Earlier genus *Trabala* Walker was treated in the family Bombycidae, however, Moore in 1883 placed it as a distinct genus in the family Lasiocampidae. (Hampson, 1892; Holloway, 1987; Zolotuhin, Treadaway & Witt, 1997; Zolotuhin & Witt, 2000; Zolotuhin & Pinratana, 2005; Youqiao & Chunsheng, 2006, as well as, in this present study the same nomenclature has been followed. (Sujata et al, 2019) described three species of this genus from North-West India. Presently, this genus is represented by twenty-eight species from Oriental and Palearctic regions.

Species: *Trabala vishnou* (Lefèbvre, 1827)

Gastropacha vishnou Lefèbvre, 1827, Zool. J. 3: 207.

Trabala vishnou Lefèbvre: Moore, 1883, *Lepid. Ceylon,* 2: 146; Zolotuhin & Witt, 2000, *Lasiocampidae Vietnam,* 3 (11): 48; Zolotuhin & Pinratana, 2005, *Lasiocampidae Thailand,* 4: 42-44; Youqiao and Chunsheng, 2006, *Fauna Sinica,* 47: 336-338;

Zolotuhin & Ihle, 2008, *Lasiocampidae Laos,* 20(4): 3; Hauenstein et al., 2011, *Lasiocampidae Bhutan;* 67: 29; Sujata et al, 2019, *Ann. Entomol.*, 37(1): 24-26.

Trabala vishnu Hampson, 1892, *Moths India,* 1: 421-422.

Type locality. Unknown

Distribution. Throughout India; Cambodia; Central and Southern China; Malaysia; Myanmar; Nepal; North- East Pakistan; Sri Lanka; Taiwan; Thailand; Vietnam.

Remarks. In India, Nepal, and Southern China, the current species is a serious polyphagous pest of orchards and ornamental plants. Barringtonia (Lecythidaceae), Lagerstroemia (Lythraceae), Shorea (Dipterocarpaceae), and Schleichera are all known defoliators (Sapindaceae). During the present study, the ultrastructure details of the eggs of *Trabala vishnou* (Lefèbvre, 1827) have been studied for the first time

Scanning electron microscopy studies

After collection of adults from vertical sheet light traps, the freshly laid eggs by the gravid female moths are hand-picked with the help of fine forceps and brushes. The collected eggs were then preserved in glass vials containing 70% alcohol and glycerol at a ratio of 8:2. The vials were marked with proper reference numbers and field data such as name of locality, collector's name, and date of collection.

To prepare samples for scanning electron microscopy studies, the following procedure was adopted:

The sample substance i.e., the eggs were fixed in 2.5% glutaraldehyde for a minimum duration of one hour. Then, the material was shifted into phosphate-buffered saline (PBS) at pH 7.4 and rinsed repeatedly for a minimum period of 15 min.

The eggs were dehydrated by maneuvering a succession of graded ethyl alcohol (in 50% alcohol for 15 min, followed by 70% and 90% alcohol for 15 min each and 3 changes in 100% alcohol for 10 min each).

After proper dehydration and drying of sample material in air it was mounted on aluminium stubs with double-sided adhesive carbon tape and sputter coated with a mixture of gold and platinum.

The sputtered samples of the eggs were observed and studied through a scanning Electron Microscope (JEOL) JSM-6510LV available in the Sophisticated Instrumentation Centre of Punjabi University, Patiala. The sample material was scanned under this microscope to assess the chorion, micropylar region, the arrangement of micropylar rosettes, aeropyles and other external ultra-structures present on the eggshell.

The eggs were classified according to the nomenclature systems proposed by (Doring, 1955; Peterson, 1964). The terminology used by Salked, 1975; Zolotuhin & Kurshakov, 2009; Korycinska, 2012 and Dolinskaya, 2019 was been followed in this study.

RESULTS

Capissa vagesa (Moore, 1859)

Egg shape: In this species, the eggs are spherical in shape with 0.826mm length and 0.6mm width (Fig. 1a).

Egg chorion: The exo-chorion of the egg is smooth and very lightly sculptured with a plain surface area. It has very fine, thin and almost faded lines all over the surface. It forms an intricate, exquisite patterned polygonal cell arrangement comprising majorly of hexagonal and heptagonal shaped polygons. These polygonal cells are without any raised or thick boundaries and are joined by imaginary lines connecting at each corner with an aeropylar opening. No depressions, groves, pits, or rough texture are observed on the egg surface. After the transition area, the exo-chorion shows smooth and texture-free surface up to the basal area of the egg (Fig. 1b).

Aeropyles: Air cavities are present on each corner of the polygonal cells on general exo-chorionic surface area except micropylar and basal regions of the egg. The aeropyles are very minute, rounded and without any distinct boundaries on the exo-chorionic surface and present at each corner of the hexagonal and heptagonal cells. The number of aeropyles is in accordance with the shape and size of the polygonal cells and their number ranges from 6-7 (Fig. 1c).

Micropylar region: In this species, the micropylar region is very prominent and clearly visible in the center at the anterior pole of the egg. The micropylar region consists of micropylar openings and micropylar rosettes with primary and secondary petaloid cells (Fig. 1d).

Micropylar rosette: The micropylar rosette is composed of 9 to 11 primary petaloid cells and only one petaloid cell is smaller in size than others forming asymmetric arrangement in the rosette. The primary cells surround the micropylar pit and are observed with very fine and slightly raised boundaries in side view. The boundaries of these cells are fused with each other up to 2/3rd of their length from where the curves start forming. The internal area of these cells is slightly textured. The primary cells are further surrounded by a row of secondary petal shaped cells, which seem fading to form the transitional zone i.e., to demarcate the end of the micropylar region (Fig. 1e).

Micropyles: These openings are present at the center of the micropylar rosette. In this study, it has been revealed that the number of micropylar openings always depends on the number of primary cells that surround the micropylar pit **(**Fig. 1f).

In the eggs having 9 primary petal shaped cells, the micropylar pit plus-shaped and 4 micropyles are present, each at the corner end of plus sign. Whereas, in the eggs having 11 primary petal-shaped cells, the micropylar pit is star-shaped and has 5 micropylar openings, each present at the corner end of the star. In both cases, the micropylar pit is somewhat depressed and present at the center of the rosette having minute rounded micropylar holes. No sign of prominent thick boundaries is seen (Fig. 1f).

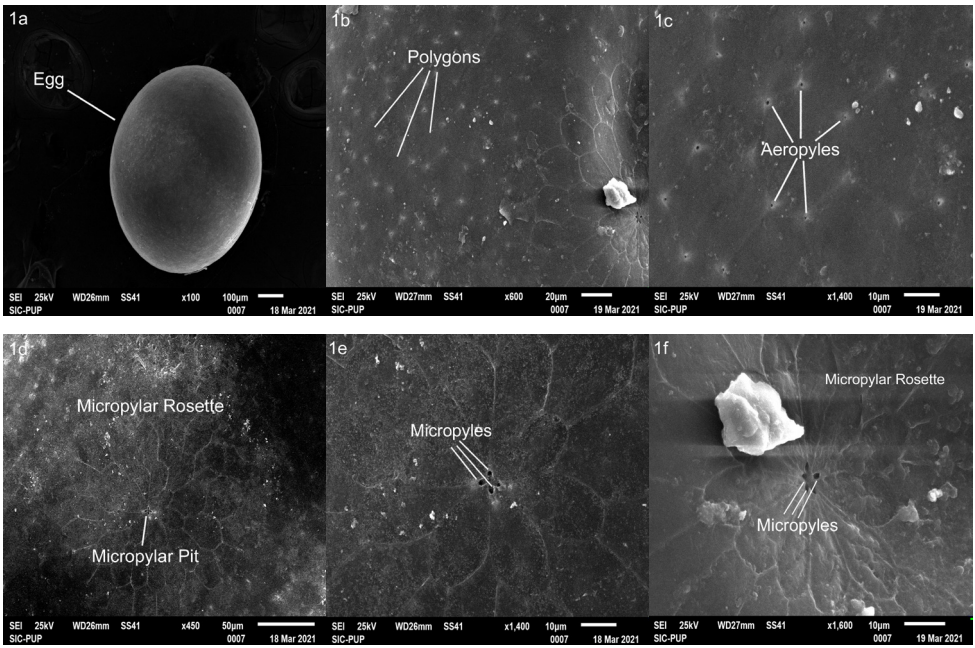

Figure 1. SEM of *Capissa vagesa* (Moore, 1859). a) dorsal view (egg), b) egg chorion, c) aeropyles, d-f) micropylar rosette).

Trabala vishnou (Lefèbvre, 1827)

Egg shape: In this species, the eggs are oval with 2.418mm length and 1.860mm width. Eggs are covered with complexly threaded flagellate hairs of the female moth's abdomen i.e., anal tuft. These hairs form a nest-like covering at the base or posterior pole of the egg and cover half of the egg surface toward its anterior pole. The general surface of the egg is with a highly sculptured pattern, which is visible under magnification SEM (Fig. 2a).

Hair-tufts: The female abdominal hairs present on the base are straight and flagellated with pointed tip endings in structure and aligned in an irregular fashion of crisscrossing. These are part of the anal tuft and function as a protective layer for egg from harsh and unfavorable environmental conditions (Fig. 2b).

Egg chorion: The chorion is highly sculptured and patterned with pentagonal, hexagonal and heptagonal cells all over the egg surface. These polygonal cells are with very thick and uniform boundaries. These boundaries are slightly raised above the egg surface area and form crater-like depression in the center of each polygonal cell. The aeropylar openings are present on thick boundaries of each polygonal cell circumscribing the polygonal crater and their number varies as per the shape of the cell (Fig. 2c).

Aeropyles: The aeropylar openings vary in number according to the shape of the polygonal cells. Aeropyles are present at the corners of each polygon. In heptagonal cells 7 aeropyles are present and their number in hexagonal and pentagonal cells is 6 and 5 respectively (Fig. 2d).

The structure of the aeropyles is simple having tiny holes with minutely visible bordering depression at the center of thick boundaries of polygonal cells, which further led to the aeropylar canals under the chorion layer (Fig. 2d).

Micropylar region: While examining the anterior pole of the egg under a scanning electron microscope, no usual area was revealed as in the earlier studied egg. No distinct micropylar region is present at the anterior end of the egg; rather it is a smooth surface showing no signs of micropylar region and its other important parts such as prominent micropylar rosette and openings. It shows no distinct structure which can be compared to a micropylar region of a lepidopteran egg (Fig. 2e).

Even all the sculptured cells and their thick boundaries become quite smooth the anterior pole of the egg. The aeropyles are also absent in the upper 1/3rd part of egg toward anterior end (Fig. 2e).

Micropyles: As discussed above, no unique structure is present at the anterior pole of the egg in this species. On thorough examination, only two prominent, deep and nearly round openings are observed almost in the center of the assumed micropylar region at the anterior pole of the egg. These two small openings are present one cell apart from each other, in the center of the craters formed on the chorion surface, circumscribed by thick and raised uniform boundaries. These openings are clearly distinct from the aeropylar openings. These points are sufficient to prove their identity as micropylar openings (Fig. 2f).

These two openings are bigger and not fully round or circular in their shape as observed in aeropyles.

These openings are present in the centre of depression or crater of the cells surrounded by thick boundaries. Whereas, in the case of aeropyles, it is clearly observed that the aeropylar openings are present on the thick boundaries of the cells and a total 5-7 per cell at each corner. This is not the case with these two micropylar openings.

Therefore, leaving aside all the important structures of a micropylar region that are expected to be present in the lepidopteran eggs, it has been concluded that the eggs of this species possess only a smooth micropylar plate and have only two micropylar openings adjacent to this plate for aiding entrance to sperms for fertilization.

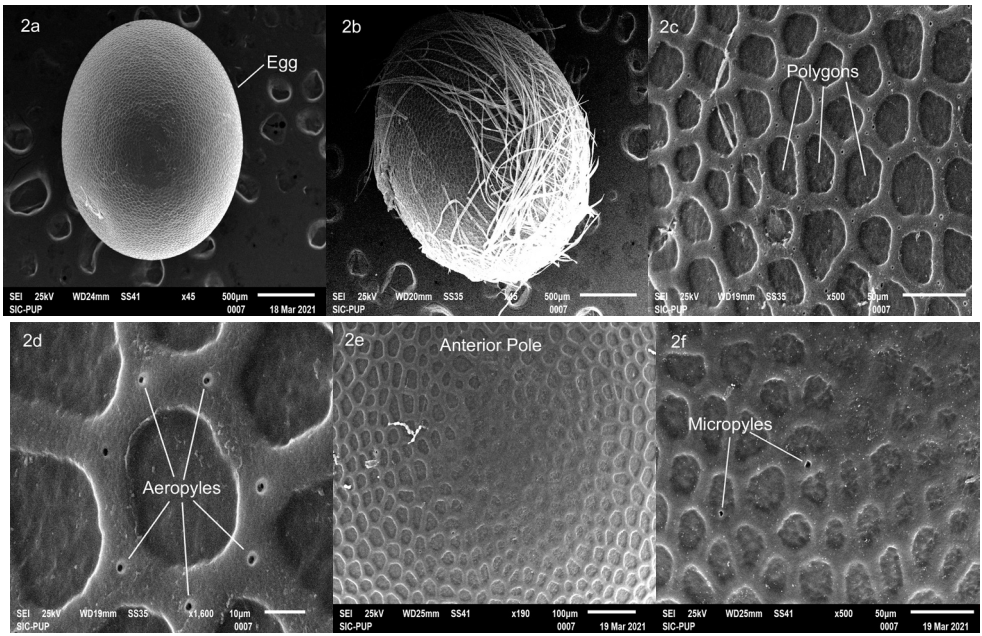

Figure 2. SEM of *Trabala vishnou* (Lefèbvre, 1827) a) dorsal view (egg), b) lateral view (egg with hairs), c) chorion, d) aeropyles, e) micropylar region, f) Micropyles.

CONCLUSIONS AND DISCUSSION

The egg shape, anatomy of the egg chorion, and distinct chorion patterns in two species of moths, *Capissa vagesa* (Moore, 1859) and *Trabala vishnou* (Lefèbvre, 1827), were explored and studied using a scanning electron microscope in this study. In both the studied species, the exterior morphology or ultrastructure of the egg's chorion is unique in terms of chorion sculpturing, the number of primary cells forming the micropylar area, shapes of polygonal cells, number of micropylar and aeropylar apertures present on the chorion surface.

An attempt has also been made to summarize the ultrastructural details of the eggs of these two species under study for comparison purposes in this paper as (Table 1.).

Table 1. Comparative account of important ultrastructural egg characters between studied species.

Species	Egg features								
	Egg Shape	Chorion Texture	Shape of Polygons	Number of Aeropyles (per polygonal cell)	Number of Micropyles	Primary Petaloid Cells (PPC)	Secondary Petaloid Cells (SPC)	Shape of Micropylar Pit	Egg hairs or Setae
Capissa vagesa (Moore, 1859)	Spherical	Very lightly sculptured	Hexagonal & Heptagonal	6-7	4-5	8+1, 10+1 Petaloid	Present	Plus & Star Shaped	-
Trabala vishnou (Lefèbvre, 1827)	Oval	Highly sculptured	Pentagonal, Hexagonal & Heptagonal	5-7	2	-	-	-	Present

The eggs of *Capissa vagesa* (Moore, 1859), revealed the general pattern of egg chorion as found in other representatives of family Erebidae as investigated by (Doring, 1955; Peterson, 1963) by light microscopy. The egg shape of this moth is observed as spherical when seen dorsally which resembles to the eggs of other species referable to the family Erebidae.

In *Trabala vishnou* (Lefèbvre, 1827), its egg chorion unveils altogether unusual observations compared to other eggs of lappet moths (Zolotuhin & Kurshakov, 2009). Usually, a Lasiocampid egg shell shows a full-fledged micropylar structure with other important parts such as micropylar pit with micropyles and micropylar rosette at the anterior top of the egg (Zolotuhin & Kurshakov, 2009). But in the present species i.e., *Trabala vishnou* (Lefèbvre, 1827), its egg showed no distinct micropylar region at the top anterior end. Three egg samples have been investigated under a scanning electron microscope. Only two tiny holes i.e., micropyles, have been observed at the anterior pole and observed no other micropylar structure. The egg chorions of this species have been scanned and investigated for the first time.

Kawaguchi, Banno, Koga, Kawarabata, & Doira (1996) and Wolf, Murphy, Reid, & Garraway (2000) concluded that the number of micropylar openings present in the center of a micropylar rosette in the eggs has a fixed relationship with the shape of the micropylar pit. They observed that in eggs having a star-shaped micropylar pit, the number of micropyles is usually 5-6 and in case of eggs with a cross or plus-shaped micropylar pit, they are usually 4 in number. According to which the total number of primary petaloid cells also varies in its number and formation. In the present study, the same has been observed in *Capissa vagesa* (Moore, 1859) of family Erebidae.

It has been observed that the egg with a micropylar rosette of 9 petaloid cells with a cross or plus-shaped micropylar pit, possesses 4 micropylar openings at each corner of the cross and the other egg with a micropylar rosette of 11 petaloid cells with a star-shaped micropylar pit, have 5 micropylar openings at each corner of the star. This is in accordance with the earlier observations made by (Wolf et al*,* 2000) in the case of *Utetheisa ornatrix* (Linnaeus, 1758) referable to family Erebidae and (Kawaguchi et al*,* 1996) in case of *Bombyx mandarina* (Moore, 1872) of the family Bombycidae.

As far as the information about aeropylar openings present on the egg chorion in lepidopteran eggs is concerned, quite limited studies are there (Fehrenbach, Dittrich & Zissler, 1987) attempted to count the number of aeropyles present on egg chorions in two Noctuid species, namely *Heliothis virescens* (Fabricius, 1777) and *Spodoptera littoralis* (Boisduval, 1833). They recorded 50 aeropyles per egg in *Heliothis virescens* (Fabricius, 1777) and 400 aeropyles per egg in *Spodoptera littoralis* (Boisduval, 1833). In this study, an attempt has been made to examine the number as well as shape of aeropylar openings in the species being studied. The number of aeropyles per polygonal cell present on the egg chorion has been recorded and revealed variance in their number in these two species of moths under study, the number of aeropyles per polygonal cell observed in the eggs of *Capissa vagesa* (Moore, 1859) are 6-7 which were minute faded circles in shape whereas, on the egg chorion in case of

Trabala vishnou (Lefèbvre, 1827), they were limited to approximately 5-7 deep and thick round circles per polygonal cell.

For identification and distinction of discrete species, superficial morphological traits as coloration, ornamentation of the head, thorax, and abdomen, wing maculation, wing venation, male and female genitalic characters are traditionally used. Arbogast et al (1980) described the ultrastructural and morphological details of chorionic sculpturing of the eggs in 10 species of stored product moths referable to families Pyralidae, Gelechiidae and Tineidae. They discussed about pattern of sinuous ridges or carinae and cells, structure of aeropyles and texture of chorion on cell discs and also formulated a taxonomic key for identification of eggs on the basis of these characters. Likewise, based on the current findings, it is easy to assume that the ultrastructural traits are likewise noteworthy, and that they can authenticate and reinforce the morpho-taxonomy.

Korycinska (2012) studied and illustrated the eggs of seven economically important species of moths i.e., *Autographa gamma* (Linnaeus, 1758), *Helicoverpa armigera* (Hübner, 1808), *Lacanobia oleracea* (Linnaeus, 1758), *Mamestra brassicae* (Linnaeus, 1758), *Spodoptera exigua* (Hübner, 1808), *Spodoptera littoralis* (Boisduval, 1833), *Spodoptera litura* (Fabricius, 1775) of family Noctuidae. He also formulated a taxonomic key for identification of these species for their easy quarantine and management during trades. It confirms the importance of this study not only for morphological identification purposes but also in sector of pest management for early detection of important pest species through means of egg characters.

With naked eyes, it is difficult to distinguish between closely related species based on morphological traits such as form, size, color, and texture of the eggs. That is why, for adequate distinction, additional well-founded taxonomic features must be included. Ultrastructure examinations on many characteristics such as shape, chorion texture and pattern, and other structural details of eggs of these two species of ditrysian moths were carried out for this purpose. Further examination of such traits appearing on the eggs of other moth species is required. These findings will add important facts and details to moth taxonomy and phylogenetic studies, as well as be useful for the delimitation of closely related species.

From India, Kumar et al (2002) compiled the fine ultrastructural details of chorion and shell layers of the eggs of a snout moth species namely *Diaphania pulverulentalis* Hampson, 1896 of family Pyralidae. They observed recognizable crisscross net-like pattern of rectangular and pentagonal cells on the chorion. They also recorded asymmetrical micropylar rosette with 8 dissimilar petal-fashioned cells and 15-18 ring-shaped aeropylar openings per egg. Kumar, Rajadurai, Babu, & Kariappa (2003) investigated the ultrastructural details of chorion of the eggs of a lepidopteran pest of Mulberry i.e., *Amata passalis* (Fabricus, 1781) of family Amatidae The eggshell structure of the pest species revealed highly adorned chorion structure with micropylar rosette of 15-19 petal-shaped primary cells. The general chorion surface was seen with a reticulate pattern of pentagonal and hexagonal cells and each cell mounted

with 3-6 aeropyles of 0.36±0.008mm diameter. Kumar, Kariappa, Babu, & Dandin (2007) examined the surface ultrastructure of the eggshell of Eri silkworm *Samia ricini* (Boisduval, 1854) of family Saturniidae by using scanning electron microscope and studied the chorion characters such as micropyles, micropylar rosette, micropylar canals, shell imprints and aeropyles.

At last, in support of our study we like to conclude that, a precise benefaction, in terms of improving and elevating the morphological personation of various moth families, has been carried in the current work. An attempt has been made to engrave these egg structures to identify these moth species. It is worth repeating that SEM inspections of these moth eggshells will show them to be an advanced method. Such studies can aid in the differentiation of distinct taxa at the family, genus, and species levels, as well as in the resolution of species complexes. As a result, this study will undoubtedly provide a firm basis and provide a viable paradigm for future researchers undertaking similar studies on Indian moths.

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Isolation and Characterization of Bacterial Gut Symbionts from Irradiated, Wild and Lab Reared Males of Melon Fly, *Zeugodacus cucurbitae* (Coquillett)

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ABSTRACT

The melon fly, *Bactrocera cucurbitae* is of serious concern inflicting heavy losses to the horticultural industry. It attacks cucurbits right from the primordial stages of the crop up to harvest and causing yield loss of 30 to 100%. The Sterile Insect Technique is a species-specific and environmentally non-polluting method of insect control that relies on sterilization of males using gamma rays and systemic release of sterile males in wild environment. Male pupae of *Zeugodacus cucurbitae* were exposed to gamma radiation at 50 Gy using Cobalt-60 source. Bacterial gut symbionts from sterile males, wild males of field collected population and laboratory reared males of melon fly were isolated and characterized based on morphological characteristics (*viz.,* colour, size, shape, opacity, margin, elevation and viscosity), gram staining, morphology and arrangement of bacterial cells in culture media. Ten adult flies from sterile, wild and laboratory reared males were used for isolation of gut symbionts. The most dominant phylum of bacteria found among the sterile, wild and lab reared male flies was Proteobacteria followed by the phylum Firmicutes. Different genera of bacteria isolated from sterile males were *Enterobacter, Providencia* and *Bacillus.* From wild males; *Enterobacter, Providencia, Morganella, Klebsiella* and *Bacillus* were identified. Bacterial genera obtained from lab reared males were *Enterobacter, Providencia, Klebsiella* and *Bacillus.* Among the entire bacterial genus, *Enterobacter, Providencia* and *Bacillus* were the common bacterial genera isolated from sterile, wild and lab reared male flies. Irradiation had resulted in loss of endosymbiotic bacteria in sterile males.

Keywords: Melon fly, wild males, lab reared males, sterile males, gamma radiation, bacterial gut symbionts.

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INTRODUCTION

The Melon fly, *Zeugodacus cucurbitae* (Coquillett) (Tephritidae: Diptera) is one of the world's most economically important horticultural and quarantine insect pests causing significant losses to the horticultural industry (Vargas, Pinero, & Leblanc, 2015). It is found worldwide, but India is considered to be its native habitat. Melon fly is a serious pest on cucurbits but it has also been found in beans, tomatoes and other commercial crops. It attacks 81 plant species belonging to 19 different families and destroys more than 60% of cucurbit crops in India (Kapoor, 2005). Cucurbits are subjected to damage by Melon fly right from the primordial stages of the crop up to harvest and yield loss due to fruit fly damage varies from 30 to 100%, depending on the population of fruit fly and cultivar type (Viraktamath, Mallik, Chandrashekar, Ramakrishna, & Praveen, 2003). The insect-bacterial association between hexapod and gut bacteria has complex interactions and is essential in providing natural sources of nitrogen, amino acids and vitamins which are lacking in host fruits, gut physiology and reproduction (Douglas, Minto, & Wilkinson, 2001). The microbiota of *Enterobacteriaceae* were found to influence several biological traits of Med fly, such as shortening of immature developmental stages, increasing fecundity, extending survival, increasing male mating competitiveness and increasing female mating receptivity (Deutscher, Chapman, Shuttleworth, Riegler, & Reynolds, 2019). Volatiles produced by the microbial symbionts play a crucial role in insect communication and production of pheromone. The sex pheromones trimethylpyrazine (TMP) and tetramethylpyrazine (TTMP) produced in the male rectum of *Bactrocera dorsalis* (Hendel) showed a strong attraction to wild females (Ren, Yingao, Mingxue, Yongyue, & Cheng, 2021). The bacteria within the intestine of adult flies have crucial functions in host development and reproduction and they significantly contribute to overall host fitness (Ben-Yosef, Jurkevitch, & Yuval, 2008). Endosymbiotic bacteria also known to influence host gut physiology, tissue homeostasis and environmental stress tolerance, as well as host resistance to pesticides and pathogens (Broderick, Buchon, & Lemaitre, 2014; Cheng et al, 2017).

Insect arthropods have a symbiotic relationship with microorganisms particularly bacteria as endosymbionts. Microbial symbionts play an important role in biology of many insects influencing insect nutrition, immunity, reproduction, ecology and evolution. Fruit flies have a variety of bacterial symbionts in their digestive system influencing a variety of developmental and fitness parameters. This functional contribution of symbiotic microorganisms to insect physiology could be useful in mass-rearing facilities. Bacterial strains isolated from microbiota of wild individuals can be given as supplements to mass reared insects in an attempt to replicate the natural microbiome and improve fitness and mating success (Ras, Beukeboom, Caceres, & Bourtzis, 2017). Mass rearing and irradiation of male flies may undermine the quality and mating vigour of the flies due to adverse effects of gamma radiation. Isolation and identification of beneficial bacterial endosymbionts from wild males of fruit flies will helpful in improving the fitness of irradiated and lab reared and males by providing in the form of probiotic diets (Hamden, Guerfali, Fadhl, Saidi, & Chevrier, 2013; Kyritsis, Augustinos, Caceres, & Bourtzis, 2017).

The process of radiation may undermine the quality of sterile males by affecting their mating vigour and competitiveness. Furthermore, the poor fitness and field performance of sterile males caused by irradiation may be associated with injured tissues and condition of the bacterial symbionts of their digestive system (Lauzon & Potter, 2012). The bacteria present in released males after mass rearing and irradiation may differ from their wild counterparts which impair their mating performance. Thus, restoring key symbiotic bacteria in mass-reared sterile flies prior to release is essential for improving the efficiency of Sterile Insect Technique (SIT). Providing symbiotic bacteria in pre-release adult holding diet in the form of probiotics improves the sexual performance of the released sterile males in the field. The use of semiochemicals and probiotic treatments increased the competing ability of sterile males in fruit flies (Hamden et al*,* 2020). Hence, it is necessary to know the bacterial communities in Melon fly to enhance the effectiveness of male sterile insect technique. The present work was therefore undertaken to identify and characterize the gut bacterial communities of irradiated, wild and lab reared males of *Z. cucubitae* using culture dependent approach.

MATERIALS AND METHODS

The studies on isolation and characterization of bacterial gut symbionts of *Z. cucurbitae* was conducted during 2021-22 at Post Graduate (PG) research laboratories of Department of Entomology and Plant Pathology, S.V. Agricultural College, Tirupati, India.

Pupae required for irradiation were raised by rearing larvae of Melon fly on larval liquid diet (Panduranga, Sharma, & Sharma, 2018). The male pupae of 48 hours before adult eclosion were packed carefully in plastic petri plates and exposed to gamma radiation (Cobalt60 source) at 50 Gy using gamma chamber (GC- 5000, BRIT and AERB) at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka. Wild flies were obtained from the Melon fly infested bitter gourd (*Momordica charantia* L.) fruits from field. Infested fruits were brought to the laboratory and were kept at controlled conditions (25-27°C, 65-75% RH) in 8×6″ glass jars provided with 5 cm thickness of sterilized sand for pupation. The fully-grown larvae popped out from the fruit into soil for pupation. Pupae were collected and transferred to adult rearing cages (30×30×30cm). Adults were provided with a mixture of sugar and yeast hydrolysate (3:1) as adult diet and water-soaked cotton swabs in 100 ml conical flask as source of water. Cages were cleaned and replaced with adult diet and water as and when necessary. Laboratory reared male flies were obtained by providing natural ripened pumpkin host fruits to initial Melon fly culture maintained in the laboratory for oviposition. Pumpkin fruits oviposited by melon flies were collected and transferred into plastic rearing trays (60×40×7.5cm) with 5 cm thickness of sterilized soil and the trays were covered with black cotton cloth secured by rubber band. Ripened pumpkin fruits were provided to the developing maggots on alternate days. The fully-grown larvae popped out from fruit into soil for pupation and pupae were collected and transferred to adult rearing cages (30×30×30cm). Adults were provided with a mixture of sugar and yeast

hydrolysate (3:1) in small petri plates as adult diet and water-soaked cotton swabs in 100 ml conical flask as source of water.

Ten adult male flies from sterile, wild and laboratory reared *Zeugodacus cucurbitae* were used for isolation of bacterial gut symbionts by following procedures described by Lloyd, Drew, Teakle, & Hayward (1986). Sterile, wild and laboratory reared live male flies were taken separately in glass vials and kept in a refrigerator at 5°C for 5 min to cold-anesthetize and to prevent regurgitation of gut fluid. Before dissection of flies, the flies were surface sterilized by immersing in 70% ethanol for 30 seconds and then flies were dipped in 0.25% sodium hypochlorite for 1 minute. Finally flies were washed thrice with distilled water to remove the external contamination of microorganisms. The surface-sterilized male flies were individually dissected in sterile agar-agar plates under laminar air flow using a stereomicroscope (Olympus SZ 61 with magnification of 5x). The dissected alimentary tract's midgut (Fig. 1) was carefully separated and squeezed with a sterile glass rod (Tauc, Tasdogan, & Pandur, 2014).

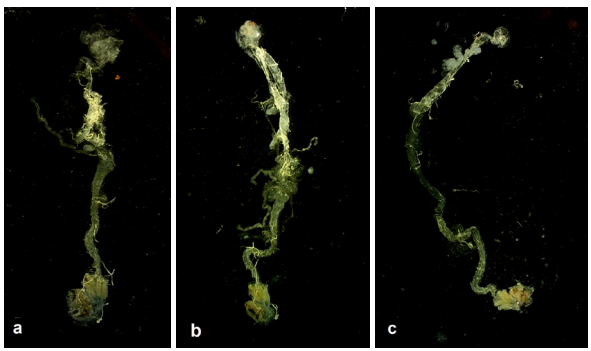

Figure 1. Dissected gut sample from a) sterile male, b) wild male, c) lab reared male of *Zeugodacus cucurbitae*

A loopful of squeezed gut fluid from sterile, wild and lab reared males were streaked separately on Nutrient Agar (NA) media in different petri dishes and were incubated for 24-48 hours in BOD incubator at 34°C. After 14-days, predominant bacterial isolates from each culture were obtained through repeated sub-culturing to obtain pure culture. The purified individual bacterial isolates were preserved in agar slants for further characterization (Fig. 2).

For morphological characterization of bacterial gut symbionts; purified bacterial isolates were screened based on colony characteristics (*viz.,* colour, size, shape, opacity, margin, elevation and viscosity), Gram staining, morphology and arrangement of cells. Obtained isolates were identified using Bergey's Manual of Determinative Bacteriology (Whitman et al, 2012). Gram staining technique was used to differentiate Gram-positive and Gram-negative bacteria. A Gram-positive bacterium stains purple, while Gram-negative bacterium stains pink or red when subjected to Gram staining. The Gram-staining technique was performed by following the method of Claus (1992). The shape and arrangement of bacterial colonies were obtained by capturing images

using Olympus CX 41 microscope equipped with a Magcam DC 5 digital camera (5.1 MP, 1/ 2.5″ CMOS sensor) at the magnification level of 100x /1.25 oil**.** The length and width of the spores of all the bacterial isolates were also measured using the above mentioned microscope. Colony characteristics *viz.,* colour, size, shape, opacity, margin, elevation and viscosity of the pure isolates colonies were observed under light microscope.

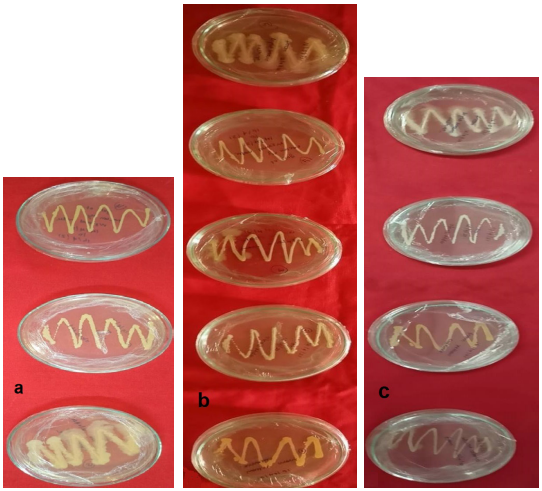

Figure 2. Sub cultures of bacterial isolates from gut samples of a) sterile, b) wild, c) lab reared males of *Zeugodacus cucurbitae*.

RESULTS

Morphological characteristics of bacterial gut symbionts isolated from sterile males of melon fly (BCS) are presented in table 1 and depicted in fig. 3. Colony and cell morphology of three different isolates were recorded.

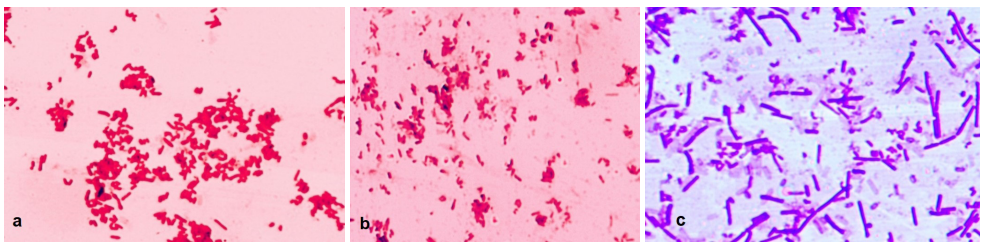

Figure 3. Morphological characterisation of bacterial gut symbionts from sterile males of *Zeugodacus cucurbitae*. a) *Enterobacter*, b) *Providencia*, c) *Bacillus*.

Colonies of BCS1 (Bacterial culture of sterile males) isolate were Gram-negative, rod shaped, large, flat, translucent, dull yellow colour with raised and convex elevation and irregular edges. BCS2 isolate were large, circular, translucent, moist, creamy white colour with convex elevation and entire margin. Isolate of BCS3 colonies were

Gram-positive, rod shapes, large, circular, opaque, dry, white colour with flat elevation and irregular margin (Table 1). Based on morphological characteristics, the isolates such as BCS1, BCS2 and BCS3 obtained from the gut of sterile male flies were identified as *Enterobacter, Providencia,* and *Bacillus,* respectively (Table 2).

Table 1. Morphological characterization of bacterial gut symbionts from sterile males of *Zeugodacus cucurbitae*

Isolates	Colony morphology							Gram staining	Cell morphology		Cell arrangement
	Size	Shape	Opacity	Texture	Elevation	Colour	Margin		Size (µm)	Shape	
BCS1	Large	Flat	Translucent	Mucoid	Raised	Dull yellow	Irregular	Negative	0.75 X 1.72	Rod	Single
BCS2	Large	Circular	Translucent	Moist	Convex	Creamy white	Entire	Negative	0.73 X 1.79	Rod	Single and pairs
BCS3	Large	Circular	Opaque	Dry	Flat	White	Irregular	Positive	1.13 X 5.7	Rod	Single

BCS1-Bacterial culture from sterile males isolate 1; BCS2-Bacterial culture from sterile males isolate 2; BCS3-Bacterial culture from sterile males isolate 3.

Table 2. Bacterial gut symbionts from sterile males of *Zeugodacus cucurbitae.*

Isolate	Identification	Family	Phylum
BCS1	Enterobacterbacter	Enterobacteriaceae	Proteobacteria
BCS2	Providencia	Enterobacteriaceae	Proteobacteria
BCS3	Bacillus	Bacillaceae	Firmicutes

BCS1-Bacterial culture from sterile males isolate 1; BCS2-Bacterial culture from sterile males isolate 2; BCS3-Bacterial culture from sterile males isolate 3.

Morphological characteristics of bacterial gut symbionts isolated from field collected population (wild male flies) of melon fly (BCW) are presented in table 3 and fig. 4. Five bacterial isolates were obtained from wild male flies were observed for colony and cell morphology. Colonies of isolate BCW1, BCW2, BCW3 and BCW4 (Bacterial culture of wild males) are Gram-negative, rod shaped bacteria cells that were large, flat, translucent. Colour of the colonies of BCW1, BCW2, BCW3 and BCW4 are dull yellow, creamy white, opaque and white in colour, respectively with raised and convex elevation. Colonies of BCW5 were Gram-positive, rod shaped single cells, large, circular, dry, white colour with flat elevation and irregular margin (Table 3). Based on the characteristics of the isolates; BCW1, BCW2, BCW3, BCW4, and BCW5 obtained from the gut of *Z. cucurbitae* wild males were identified as *Enterobacter, Providencia, Morganella, Klebsiella* and *Bacillus,* respectively (Table 4).

Table 3. Morphological characterization of bacterial gut isolates from wild males of *Zeugodacus cucurbitae.*

Isolates	Colony morphology							Gram staining	Cell morphology		Cell arrangement
	Size	Shape	Opacity	Texture	Elevation	Colour	Margin		Size (µm)	Shape	
BCW1	Large	Flat	Translucent	Mucoid	Raised	Dull yellow	Irregular	Negative	0.73 X 1.73	Rod	Single
BCW2	Large	Circular	Translucent	Moist	Convex	Creamy white	Entire	Negative	0.71 X 1.78	Rod	Single and pairs
BCW3	Small	Circular	Opaque	Mucoid	Convex	White	Entire	Negative	0.66 X 1.15	Rod	Single
BCW4	Large	Circular	Opaque	Mucoid	Convex	Creamy white	Entire	Negative	0.75 X 1.81	Rod	Single, pairs and short chains
BCW5	Large	Circular	Opaque	Dry	Flat	White	Irregular	Positive	1.12 X 5.5	Rod	Single

BCW1-Bacterial culture from wild males isolate 1; BCW2-Bacterial culture from wild males isolate 2; BCW3-Bacterial culture from wild males isolate 3; BCW4-Bacterial culture from wild males isolate 4; BCW5-Bacterial culture from wild males isolate 5.

Table 4. Bacterial gut symbionts from wild males of *Zeugodacus cucurbitae*.

Isolate	Identification	Family	Phylum
BCW1	Enterobacterbacter	Enterobacteriaceae	Proteobacteria
BCW2	Providencia	Enterobacteriaceae	Proteobacteria
BCW3	Morganella	Enterobacteriaceae	Proteobacteria
BCW4	Klebsiella	Enterobacteriaceae	Proteobacteria
BCW5	Bacillus	Bacillaceae	Firmicutes

BCW1-Bacterial culture from wild males isolate 1; BCW2-Bacterial culture from wild males isolate 2; BCW3-Bacterial culture from wild males isolate 3; BCW4-Bacterial culture from wild males isolate 4; BCW5-Bacterial culture from wild males isolate 5

.Morphological characteristics of bacterial gut symbionts isolated from lab reared males of *Z. cucurbitae* (BCL) are presented in table 5 and fig. 5. Colonies of BCL1, BCL2, and BCL4 (Bacterial culture of lab reared males) isolates were Gram-negative, rod shaped, single occurred cells. Colonies are larger in size, circular - flat shape, translucent with mucoid viscosity. The colour of BCL1, BCL2, and BCL4 are dull yellow (irregular margin), creamy white colour with convex elevation, respectively. Whereas BCL3 colonies were Gram-positive, rod shaped, large, circular, opaque, white colour with and irregular margin (Table 5). Based on the morphological characteristics of BCL1, BCL2, BCL3 and BCL4 isolates from the lab reared sterile males were identified as *Enterobacter, Providencia, Bacillus* and *Klebsiella,* respectively (Table 6). The loss of endosymbionts in sterile males was mainly due to the gamma radiation.

Table 5. Morphological characterization of bacterial isolates from lab reared males of *Zeugodacus cucurbitae*

Isolates	Colony morphology							Gram staining	Cell morphology		Cell arrangement
	Size	Shape	Opacity	Texture	Elevation	Colour	Margin		Size (µm)	Shape	
BCL1	Large	Flat	Translucent	Mucoid	Raised	Dull yellow	Irregular	Negative	0.74 X 1.75	Rod	Single
BCL2	Large	Circular	Translucent	Moist	Convex	Creamy white	Entire	Negative	0.73 X 1.78	Rod	Single and pairs
BCL3	Large	Circular	Opaque	Dry	Flat	White	Irregular	Positive	1.13 X 5.6	Rod	Single
BCL4	Large	Circular	Opaque	Mucoid	Convex	Creamy white	Entire	Negative	0.74 X 1.83	Rod	Single, pairs and short chains

BCL1-Bacterial culture from lab reared males isolate 1; BCL2-Bacterial culture from lab reared males isolate 2; BCL3-Bacterial culture from lab reared males isolate 3; BCL4-Bacterial culture from lab reared males isolate 4.

Table 6. Identification of bacterial gut symbionts from lab reared males of *Zeugodacus cucurbitae*.

Isolate	Identification	Family	Phylum
BCL1	Enterobacterbacter	Enterobacteriaceae	Proteobacteria
BCL2	Providencia	Enterobacteriaceae	Proteobacteria
BCL3	Bacillus	Bacillaceae	Firmicutes
BCL4	Klebsiella	Enterobacteriaceae	Proteobacteria

BCL1-Bacterial culture from lab reared males isolate 1; BCL2-Bacterial culture from lab reared males isolate 2; BCL3-Bacterial culture from lab reared males isolate 3; BCL4-Bacterial culture from lab reared males isolate 4

CONCLUSIONS AND DISCUSSIONS

In the present study, wild males of melon flies guts harboured wide range of Enterobacteriaceae members *viz., Enterobacter, Providencia, Citrobacter, Morganella, Klebsiella.* Similarly, lab reared male flies also had diverse members of Enterobacteriaceae *viz., Morganella, Enterobacter, Providencia* and *Klebsiella.* Gut bacterial symbionts belonging to the family Enterobacteriaceae in the sterile males

were *Enterobacter* and *Providencia*. *Bacillus* belonging to the family bacillaceae and phylum Firmicutes was present in the wild, lab reared and sterile males of melon flies.

The present findings are corroborated with findings of Hadapad, Prabhakar, Chandekar, Tripathi, & Hire (2016) investigated the composition and diversity of microbial community in the midgut of the wild population of Melon fly from India. The dominant species inhabiting the Melon flies midgut were from the genera *Enterobacter*, *Klebsiella*, *Citrobacter*, *Bacillus* and *Providencia*.

Similarly, Yong, Song, Chua, & Lim (2017) observed high abundance of Proteobacteria in Carambola fruit fly, *Bactrocera carambolae.* Chandler, Lang, Bhatnagar, Eisen, & Kopp (2011) also found that *D. melanogaster* guts were rich in Enterobacteriaceae. The present findings were in accordance with Thaochan, Drew, Hughes, Vijaysegaran, & Chinajariyawong (2010) who isolated bacteria from the midgut of *Bactrocera cacuminata* (Hering) and *Bactrocera tryoni* (Forgatt) collected from the field. They found that *Citrobacter freundii, Enterobacter cloacae,* and *Klebsiella oxytoca* are predominant species in both the fruit flies. Molecular and culture-based techniques by Yuval, Ben-Ami, Behar, Ben-Yosef, & Jurkevitch (2013) also showed that members of the Enterobacteriaceae *viz., Klebsiella sp., Enterobacter sp., Pectobacterium sp., Citrobacter freundii* and *Providencia stuartii* form the dominant populations in the gut of *Ceratitis capitata* (Wiedemann). Wang, Yao, Zheng, & Zhang (2014) identified *Bacillus cereus, Enterococcus faecalis, Enterobacter cloacae* and *Citrobacter freundii* from *B. dorsalis* populations.

Present finding showed that major bacterial isolates belongs to the phylum Proteobacteria and family enterobacteriaceae which is found in accordance with Yong et al. (2017) who also observed a high abundance of Proteobacteria phylum and *Enterobacteriaceae* family in *B. carambolae.* Andongma, Wan, Dong, Li, & Desneux (2015) used 454 pyrosequencing to identify the bacteria associated with different developmental stages of *B. dorsalis* and reported that Proteobacteria dominated in immature stages while Firmicutes dominated in adult stages. Predominant bacterial culture obtained from lab reared male flies belongs to the phylum Proteobacteria and Firmicutes and these findings are on par with Cox & Gilmore (2007) who found that laboratory reared *D. Melanogaster* contained higher proportions of *Enterobacter* (Garmmaproteobacteria) or *Enterococcus* (Firmicutes). Khan, Mahin, Pramanik, & Akter (2014) also performed different biochemical, Gram reaction and motility tests to identify the mid-gut bacterial community of laboratory reared pumpkin fly, *Bactrocera tau* (Walker) and identified eight genera under the family *Enterobacteriaceae.* Pramanik, Mahin, Khan, & Miah (2014) found thirteen bacterial species from *B. dorsalis* belonging to eleven genera. They were *Listeria, Citrobacter, Moraxella, Proteus, Streptobacillus, Enterobacter, Serratia, Vibrio, Aeromonas, Klebsiella* and *Moragnella.* Augustinos et al. (2015) also found three bacterial species *viz., Providencia sp., Enterobacter sp.,* and *Acinetobacter sp*. from medfly Vienna 8 strain using culture-dependent approach.

Wild flies had diverse gut bacterial symbionts when compared to lab reared and

sterile male flies, which was concurrent with findings reported by Wang, Jin, & Zhang (2011) that field collected flies had more diverse gut flora than laboratory reared male flies of *Bactrocera dorsalis*, which was due to more opportunities to feed on natural diets. Similar findings were reported by Estes et al. (2011) that lower bacterial diversity in laboratory reared flies of the olive fruit fly, *Bactrocera oleae* (Rossi), was most likely due to laboratory conditions and artificial diets containing antibiotics and antimicrobials. Whereas the gut bacterial symbionts obtained from sterile males (irradiated at 50 Gy) were few compared to wild and lab reared males which were due to effect of gamma radiation. These results were concurrent with findings of Yuval et al. (2013) who observed that the structure of *B. dorsalis* symbiome was significantly altered at radiation dose of 50 Gy.

The result from the present studies revealed that, diverse bacterial population was found in the gut of sterile, wild and lab reared males of Melon fly, *Z. cucurbitae.* The most dominant phylum found among all the three groups of melon flies was Proteobacteria. Next to Proteobacteria, phylum Firmicutes was observed in all the flies examined. In wild males, bacteria belongs to the phylum Proteobacteria was abundant. Dominant family among all the male flies was Enterobacteriaceae and only one member belongs to Bacillaceae. *Enterobacter*, *Providencia* and *Bacillus* were common bacterial genus isolated from wild, lab reared and sterile male flies. Genus *Morganella* was present only in the gut of wild melon flies and the genus *Klebsiella* was found in both wild and lab reared flies but absent in sterile males. These results were inclined with findings of Hadapad, Shettigar, & Hire (2019) who found that guts of wild and mass-reared *Z. cucurbitae* and *B. dorsalis* had diverse bacterial composition in varying degrees of abundance. The phylum Proteobacteria was more prevalent in wild *Z. cucurbitae* and *B. dorsalis* when compared to mass-reared colonies. The dominant family in the guts of both wild and mass-reared was Enterobacteriaceae. Changes in the bacterial symbiome of irradiated melon flies included a significant decrease in the number of sequences associated with *Citrobacter*, *Raoultella* and Enterobacteriaceae members (Asikamis et al, 2019). However, the molecular characterization of bacterial gut symbionts has to be done for their confirmation. From the study, it is concluded that the process of irradiation and use of an optimum radiation dose for sterilization of males is very crucial to induce desirable level of reproductive sterility in males without causing much damage to the harboured and acquired gut symbionts in adult males, as the endosymbionts play significant role in production of pheromones and longevity of male flies. Further research may be conducted on the supplements of beneficial gut bacterial isolates to enhance different quality parameters (mating compatibility, longevity, etc.) of sterile males of *Z. cucurbitae* in support of SIT application.

DECLARATION

The authors declare that they have no conflict of interest.

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Research Article
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First Record of Three Species of Genera *Panotrogus* Reitter, 1902 and *Pseudopanotrogus* Petrovitz, 1969 (Coleoptera; Scarabaeidae; Rhizotrogini) from India

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ABSTRACT

Tribe Rhizotrogini is a tribe of June beetle containing many species of agronomic importance in India. It holds major genera like *Brahmina* (Blanchard 1851), *Eotrichia* (Medvedev 1951), *Holotrichia* (Hope 1837) or *Sophrops* (Fairmaire 1887) in India. A survey based on light trap collection was conducted in various locations of Uttarakhand to check for the presence of the species. During surveys in the years 2021 and 2022, various chafer beetles were collected to observe the distribution pattern and their hosts. Among these various species, three beetle species are here reported for the first time i.e. *Panotrogus expansus* Keith, 2003, *Panotrogus pakistanus* Keith, 2002 and *Pseudopanotrogus extrarius* Keith, 2005 from Uttarakhand, India. This also represents their first record for India. Their habitus and male genitalia are provided herein to aid in identification. A complete list of species from the Himalayan region is also provided.

Keywords: Beetles, Scarabaeidae, Melolonthinae, Uttarakhand, light trap, new record.

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INTRODUCTION

Beetle diversity in Indian Himalaya account for 47.16% of the total beetle diversity of India. Their main families include Scarabaeidae, almost 10% of total beetles species form Himalaya region. Currently, Scarabaeidae has 1843 species from India; however, Himalaya occupies 983 species from it. Scarabaeidae are the most diversified family among beetles group and are primarily scavengers (recycling dung, carrion, decaying plant material) or phytophagous. Predominantly, the phytophagous species occur in four subfamilies i.e. Melolonthinae, Rutelinae, Dynastinae and Cetoniinae.

Members of the subfamily Melolonthinae Leach, 1819 (Coleptera: Sacraoibodea) are known as June beetles. They belong to eleven tribes under north-western and north-eastern Himalaya of India (Löbl & Löbl, 2016). They are significant pests of cereals, fruit crops, forest and ornamental plant and their role has been reported in almost all states of India (Chandla et al, 1998; Theurkar et al, 2012). Among these tribes, Rhizotrogini comprise about 1800 species worldwide (Lacroix, 2022). It has about 43 genera in the Palearctic region (Löbl & Löbl, 2016)**,** of which 14 occur in the Indian Himalayan regions i.e. Himachal Pradesh, Uttarakhand, Uttar-Pradesh, Sikkim, Arunachal Pradesh, Jammu-Kashmir and Ladakh.

Of these, *Panotrogus* Reitter, 1902 and *Pseudopanotrogus* Petrovitz, 1969 are also occurring in the Himalayan states. At present, *Panotrogus* occurs in India with four species *Panotrogus batillinus* Bates, 1891*, P. hirsutus* Moser, 1913*, P. inexpectatus* Keith, 2001*, P. schmidti* Keith, 2006, *Pseudopanotrogus* with seven species (*Pseudopanotrogus donckieri* Brenske, 1892, *P. kuluensis* Moser, 1919*, P. lassallei* Keith, 2010*, P. pusillus* Arrow, 1921*, P. rosettae* Frey, 1971, *P. carinifron*s (Moser, 1909) and *P. longiceps* (Moser, 1918)), namely in the Himalayan states, especially Kashmir, Himachal Pradesh, Uttara Pradesh, Sikkim and Darjeeling (Löbl & Löbl, 2016; www.catalogueoflife.org; Keith, 2009). In addition to those already reported species, we record here for the first time the presence of two species of *Panotrogus* and one of *Pseudopanotrogus* from Uttarakhand, thus representing their first record for Indian species biodiversity.

Finally, a brief description of these species is provided based on their available specimens and photographs, to make this data available for relevant people who can contribute to the biodiversity

MATERIAL AND METHODS

Data sampling

For the collection of adult beetles, surveys were conducted in different locations of Uttarakhand, Northern state of India, during the summer of 2021-2022 with the aim to collect new species and determine their distribution pattern. The locations of surveys lay between 30.0668^0 N and 79.0193^0 E (Fig. 1, Table 1). During May and June of the respective years, when the adult beetles emerge from the soil during dusk and settle on the nearby trees to feed and mate, a strong mercury vapour lamp light trap

(150w) was installed in aforesaid locations in evening hours from 7:15 pm to 10:30 pm. Beetles were then collected and killed with ethyl-acetate and finally brought to the laboratory where they were labelled, pinned and finally placed in insect cabinet for further identification.

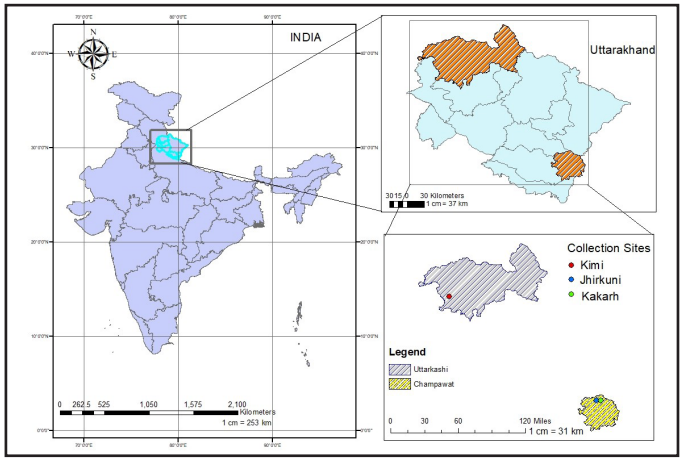

Figure 1. Map with distribution of *Panotrogus* and *Pseudopanotrogus* species in Uttarakhand based on material examined. Colour dots indicate locality in Uttarakhand, India.

Table 1. Surveyed area of white grub species during 2021-2022.

Sl. No.	Location	Altitude m.a.s.l.	Coordinates	District	Sample collected	
					♂	♀
1	Jhirkuni village Barakot	1452	29.471325°N 80.073617°E	Champawat	2	0
2	Kakarh village Barakot	1453	29.466931°N 80.061298°E	Champawat	1	0
3	Kimi village Naugaon	648	78.134392°N 30.776683°E	Uttarakashi	2	0

Among the various species of chafers, which was identified by available keys and had already been reported, few beetle species remained unidentified. They were identified using Keith 2002, 2003, 2005 keys. The males of unidentified specimens were separated and placed in 70% alcohol for genitalia extraction. Later, the genitalia and speculum gastrale were extracted carefully with forceps from the abdomen and glued on a pointed card and pinned along with the adult male specimen. The external morphological characters of genitalia of specimens were observed through Nikon SMZ745T stereo zoom microscope and adults images were obtained with Nikon D5600 digital camera while genitalia image with attached to the microscope using Leica auto montage software. Length measurements are from anterior margin of clypeus to the apices of the elytra. Photographs from the biotopes were taken with Nikon Coolpix SL9200 camera.

Study area

Uttarakhand is one of the state of India located in North West Himalaya region. At present, the total geographical area of the state is 53483 sq. km, i.e. 1.6 % of country's geographical area, out of which 46, 035 sq. km are hilly. Topographically, the state can be divided into three belts, namely the Himalaya, the Shiwalik and the Terai Region. Most of the area under state has a temperate climate, which is cold, humid, but it varies with the altitude. Its valley are found hot in summer and much cooler in winter. In the plains like Haridwar and Udham Singh Nagar climate is tropical (Chauhan, Gautam, & Negi, 2018). The annual temperature varies from 0°C to 43°C while average annual rainfall is 1550 mm.

Finally, a systematic account of the species along with material examined, description and distribution in India as well as outside India with the description of male genitalia are discussed and illustrated here.

RESULTS

Survey and collection from aforesaid locations reveal the first record of *Panotrogus expansus*, *Panotrogus pakistanus* and *Pseudopanotrogus extrarius* from India. The description of these species are as follow:

Panotrogus expansus Keith, 2003

Material examined: Uttarakhand, Champawat, Barakot, Kakarh village, 1453m, 24.06.2022, 1♂.

Distribution: Pakistan (Keith 2003) and India (Uttarakhand)) (new country record).

Male: Adult length 11.5mm (Fig. 2a).

Head: Dark reddish brown rectangular clypeus with clear sinuosity in a strongly raised incoming triangle with fine punctuation. Fronto-clypeal suture flat not evident, vertex with large punctuations bearing a distinct reclined pilosity. Antennae of 10 antennomeres including 3 on club, longer then funicle. Antennomeres 3rd, 4thand 5th elongated, 6th obconic and 7th transverse (Fig. 2b).Apical segment of maxillary palp elongated fusiform, with frosted dorsal area.

Pronotum: Anterior and posterior margins are without ciliation and base without rim. Lateral margins are crenulated with small bristle between crenulations. Punctuation anteriorly dense with reclined pilosity. Width of pronotum greatest at the middle. Anterior and posterior angles rounded, obtuse. Scutellum with punctuation and pilosity similar to the pronotum.

Elytra: Provided with uniform punctuations and reclined pilosity. Pygidium large, with pupillate, dense punctuation and erect pilosity. Protibia tridentate on the external border, the basal tooth situated towards the middle of the protibia. Apical spur inserted behind the external median tooth. Metatarsomeres with three strong teeth on the upper side, basimetatarsomere shorter than second one. Claws toothed at the base (Fig. 2c).

Aedeagus: Main branch of the parameres very thick with a fairly short obtuse secondary branch, not perpendicular to the main branch (Fig. 2d).

Figure 2. *Panotrogus expansus* Keith 2003 (Male): a) habitus, b) antennae, c) claws, d) aedeagus.

Panotrogus pakistanus Keith, 2002

Material examined: Uttarakhand, Uttarakashi, Naugaon, Kimmi village, 648m, 6.06.2022, 2 ♂♂.

Distribution: Pakistan (Keith 2002) and India (Uttarakhand) (new country record).

Male: Adult length between 10-11 mm, wholly brownish red (Fig. 3a).

Head: Clypeus rectangular, two times wider then length. Strong punctures with regular setigerous, small hairs tilted backward. Clypeo-frontal suture bisinuate and evident (Fig. 3b). Forehead slightly concave, punctuation very sparse near the suture then becoming denser on vertex.

Antennae of 10 antennomeres including 3-jointed club, longer then funicle. Antennomere 6 transverse, 7 more transverse, stretched in lamella (Fig. 3c). Apical section of maxillary palp dilated with frosted area on dorsal surface.

Pronotum: Base without rim, anterior and posterior margins glabrous. Margins crenellated with interposed cilia. Punctuation pupillate, unevenly dense, laterally especially in the anterior angles, more sparse on the disk. Pronotum widest at middle; sides converging, slightly concave anteriad, much more weakly converging posteriad, anterior angles acute and posterior obtuse. Scutellum pilosity and punctuation similar to the pronotum, except only base which is bare.

Elytra: With clear punctuation, with whitish short erect pilosity, and longer laterally. Epipleura with thin pilosity on apical third. Sternites with poorly developed smooth area. Pygidium convex with large punctuations, simple erect pilosity, long at apex, short elsewhere.

Legs: Protibia tridentate externally, latter with three equidistant dents, basal one located towards middle, apical one very strong, internal apical spur inserted in front of the median dent; metatarsomeres longer than the metatibiae with several teeth on the upper side, basimetatarsomere shorter than second one. Claws toothed at the base (Fig. 3d).

Aedeagus: ♂ genitalia lateral view (Fig. 3e).

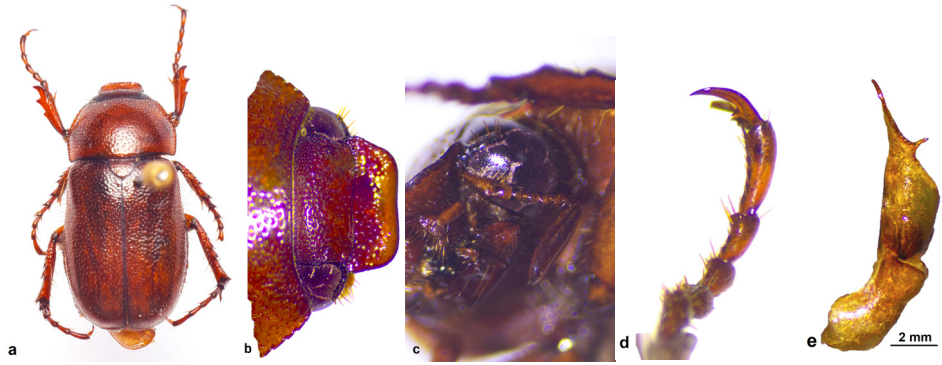

Figure 3. *Panotrogus pakistanus* Keith, 2002 (Male): a) habitus, b) clypeus, c) antennae, d) claws, e) aedeagus.

Pseudopanotrogus extrarius Keith, 2005

Material examined: Uttarakhand, Champwat, Jhirkuni village, 1452m, 23.07.2022, 2 ♂♂.

Distribution: Nepal (Keith 2005) and India (Uttarakhand) (new country record).

Male: Length almost 12mm (Fig. 4a), entirely dark brown.

Head: Clypeus apparently rectangular shape, 3 times wider than long, anterior margin slightly sinuate and strongly raised, only with sparse punctuation. Clypeo-frontal suture almost flat and evident with larger and denser punctuation set with erect pilosity. Antennae 10-jointed including a 3-jointed club, and antennomeres 6 and 7 very transverse (Fig. 4b).

Pronotum: Convex, disc bare, punctuation rather coarse, somewhat irregular, lateral and anterior margins with long cilia. Lateral margins crenelated with interspaced pilosity, greatest width of the pronotum towards middle. Scutellum smooth in the middle with margin parallel punctuations. Elytra with similar punctuation to the pronotum, integument very slightly irregular near base, pilosity microscopic on disc; epipleural ciliation made of a row of long strong hairs tapering from the base. Pygidium with dense pupillate punctuation, pilosity short, reclined, except on apical margin.

Legs: Forelegs with strong protibiae, tridentate on the external border, dents equidistant. Internal spur inserted behind the outer middle tooth; metatarsomeres longer then the metatibiae, basimetatarsomere shother than second one. Strong denticles on their upper surface. Carinae of metatibiae obsolete. Claws cleft at base (Fig. 4c).

Adeagus: It has a very special shape (Fig. 4d)

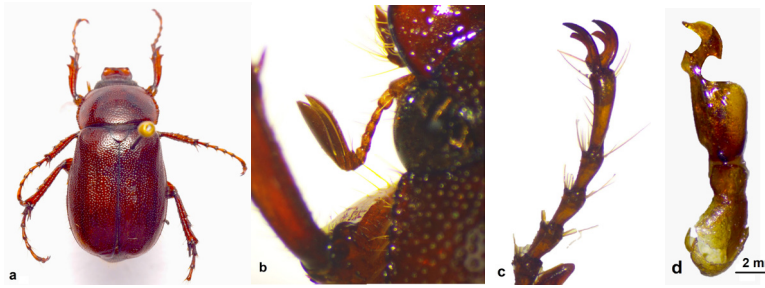

Figure 4. *Pseudopanotrogus extrarius* Keith, 2005 (Male): a) habitus, b) antennae, c) claws, d) aedeagus.

CONCLUSIONS AND DISCUSSION

In the study of genus *Panotrogus* Reitter, 1902, *P. expansus* Keith, 2003 and *P. pakistanus* Keith, 2002 were reported for the first time from India. Currently, the genus contains 22 species from Palearctic region including parts of Himalayan region of India (Löbl & Löbl, 2016). Genus *Pseudopanotrogus* Petrovitz, 1969 comprises about 13 species, mostly from the Palearctic region (www.catalogueoflife.org). They were first described by Reitter, 1902 and Petrovitz, 1969 with type species *Rhizotrogus myschenkovi* Ballion, 1871 and *Pseudopanotrogus kashmirensis* Petrovitz, 1969 respectively. Table 2 gives an account of the history of their distribution in India. With the new records of *P. expansus*, *P. pakistanus* and *Pseudopanotrogus extrarius*, both genera are now known from India by 6 and 8 species respectively.

Table 2. List of species of genus *Panotrogus* and *Pseudopanotrogus* under Himalayan states.

Sl.No	Species	Indian Distribution	References
1	*Panotrogus batillinus*	Himachal Pradesh	Bates ,1891
2	*P. hisutus*	Himachal Pradesh, Sikkim and Darjeeling	Moser, 1913
3	*P. inexpactatus*	Himachal Pradesh	Keith, 2001
4	*P. schmidti*	Uttar Pradesh	Keith, 2006
5	*Pseudopanotrogus donkieri*	Kashmir, Sikkim and Darjeeling	Brenske, 1892
6	*P. kuluensis*	Himachal Pradesh	Moser, 1919
7	*P. lassallei*	Himachal Pradesh	Keith, 2010
8	*P. pusillus*	Kashmir, Uttar Pradesh	Arrow, 1921
9	*P. rosettae*	Uttar Pradesh	Frey, 1971
10	*P. carinifrons*	Sikkim and Darjeeling	Moser, 1909
11	*P. longiceps*	Oriental	Moser, 1918

The genus *Pseudopanotrogus* can be differentiated with its strikingly elongated quadrangular clypeus, deep incised claws from apex, bald pygidium and fusiform maxillary palpus. *P. extrarius* differs from other species by its clypeus less incised in the middle. The shape of its aedeagus is also rather unique within genus *Pseudopanotrogus* (Fig. 4d). A light trap and manual scouting based survey during 2021-2022 from different locations of Uttarakhand, India revealed for the first time the presence of *Panotrogus expansus*, *Panotrogus pakistanus* and *Pseudopanotrogus extrarius* in India.

Further regular monitoring will certainly reveal more about the huge biodiversity in other unexplored parts of Uttarakhand and elsewhere.

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Bioacoustics of Tettigoniidae (Insecta, Orthoptera) Distributed in Hakkari (Eastern Anatolia) Province

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ABSTRACT

In this study, the male calling songs of sixteen species belonging to the Tettigoniidae family distributed in Hakkari were recorded in the field studies and bioacoustic analyses were performed. The bioacoustic analysis of two genera (*Kurdia* and *Novadrymadusa*) documented first time with present study and in total six species (*Bradyporus (Callimenus) latipes, Isophya hakkarica, Kurdia uvarovi, Novadrymadusa karabagi, Psorodonotus hakkari*, and *Squamiana supericola*) were presented first time. The bioacoustic analyses of seven species (*Conocephalus (Anisoptera) fuscus, Pezodrymadusa indivisa, Polysarcus zacharovi, Tettigonia armeniaca, Tettigonia caudata, Tettigonia viridissima*, and *Uvarovistia satunini*) were examined for the first time from Hakkari populations. In addition, in this study, the bioacoustic analyses of three species (*Apholidoptera kurda, Saga hakkarica*, and *Uvarovistia zebra*) distributed in Hakkari were re-evaluated in this study on new individuals. As a result of the findings, it has been determined that the male calling song of sixteen species belonging to the Tettigoniidae family are species-specific, have a simple calling song type, and morphological species hypotheses are also supported by bioacoustic data (male calling song).

Keywords: Anatolia, crickets, calling song, species hypothesis.

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INTRODUCTION

Communication in organisms can be visual, auditory, sensory or physical (contact), and communication types are generally grouped within ethological characters. Bioacoustics is a discipline of ethology that deals with sound production, song behaviour and analysis in organisms. In order Orthoptera, the family Tettigoniidae is one of the families that use acoustic communication effectively, and the male calling song is used for individuals to recognize each other, determine location, and to select the female for mate (Heller, 1988; Robinson & Hall, 2002; Heller, 2006). Since acoustic communication is the main mechanism in species belonging to the family Tettigoniidae, song characters are very important in understanding whether sexual isolation has occurred and determining the species (Heller, 2006). In most of tettigoniids, males produce calling sounds, but territorial and protest (=disturbance etc.) sounds (as pre-copulatory sounds) in some taxa (for example, *Phaneroptera*) are also known (Korsunovskaya, 2008).

In recent bioacoustic studies in Anatolia, it has been determined that the male calling song is species-specific in many taxa or species groups (Sevgili, 2004; Taylan, 2005; Sevgili, Demirsoy, & Durmuş, 2011; Şirin, Taylan, & Mol, 2014; Şirin, Taylan, Sevgili, & Mol, 2019; Taylan, Mol, Sevgili, & Şirin, 2019). Hakkari province, which is the study area, is at the south-eastern tip of the Anatolia and has borders with Iran and Iraq. Despite its high mountains and valleys, rugged topography, and high biodiversity potential, it has been one of the less-studied areas of Anatolia (Taylan, Şirin, Sevgili, & Yeşilyurt, 2021). The Orthoptera (Insecta) order is represented by a total of 741 species/subspecies in Turkey (Ünal, 2011a).

In their study on the Orthoptera fauna of Hakkari province, Taylan et al. (2021) identified 25 tettigoniid species which some of endemic or sub-endemic species for Hakkari province or Anatolia. These species are: *Isophya hakkarica* Karabağ, 1962; *Kurdia uvarovi* Karabağ, 1975; *Polysarcus zacharovi* (Stshelkanovtzev, 1910); *Phaneroptera* (*Erdemia*) *hackeri* Harz, 1988; *Anadrymadusa* (*Anadrymadusa*) *modestalis* Koçak & Kemal, 2010; *Novadrymadusa karabagi* Demirsoy, Salman & Sevgili, 2002; *Phytodrymadusa hakkarica* Karabağ, 1956; *Uvarovistia zebra* (Uvarov, 1916); *Uvarovistia satunini* (Uvarov, 1934); *Apholidoptera kurda* (Uvarov, 1916); *Apholidoptera pietschmanni* (Ebner, 1912); *Incertana persica* (Uvarov, 1917); *Platycleis intermedia* (Serville, 1838); *Saga hakkarica* Şirin & Taylan, 2019; *Squamiana supericola* (Ünal, 2018); *Psorodonotus hakkari* Kaya, Korkmaz & Çıplak, 2013; *Medecticus assimilis* (Fieber, 1858); *Tettigonia armeniaca* Tarbinsky, 1940 and *Tettigonia caudata* (Charpentier, 1845). All of these species were described as morpho-species and bioacoustic data on the species are very limited (Şirin et al, 2014; Şirin et al, 2019; Taylan et al, 2019).

In this context, we aimed to (i) test the morpho-species hypotheses of the species endemic to the region and Anatolia with bioacoustic data, and (ii) contribute to a better knowledge of bioacoustic data of Anatolian tettigoniids.

MATERIAL AND METHODS

Study area

Hakkari province (Fig. 1) is located in the southeast of the Eastern Anatolia Region, between 42° 10' and 44° 50' east longitudes and 36° 57' and 37° 48' north latitudes, and is adjacent to Iran in the east and Iraq in the south. Hakkari has a border with the internal borders of the country, the Başkale, Gürpınar, and Çatak districts of the Van province in the north, and the Beytüşşebap and Uludere districts of the Şırnak province in the western border. Hakkari city center and its surroundings are covered with mountains at different elevations and slopes. 86% of Hakkari is mountainous, 2.4% is lowland, 2.8% is the plateau, 7.5% is rough terrain and 1% is valleys (HÇŞM, 2011). Within the provincial borders of Hakkari, there are more than ten elevations over 3500 m, and the vegetation is generally East Anatolian mountain steppe, East Anatolian high mountain meadow, East Anatolian oak forest and partly wooded steppe (Gökmen, 1962; Atalay, 1983). The map of the study area was created by using the ArcGIS 10.2 (Arc Map Elevation) program.

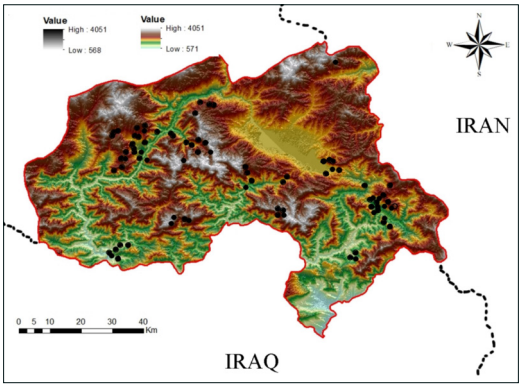

Figure 1. The map of studied area in Hakkari Provinces in this research (*black points indicate the studied localities, the map is derived from Taylan et al, 2021)

Collections, identification, and conservation of specimens

Within the scope of the study, 16 tettigoniid species were examined and male calling songs were recorded during the field studies carried out between 2020 and 2021. Considering the biological characteristics of the tettigoniids, individuals were caught with a standard sweep net at suitable localities and the coordinates of the collection points were taken with the help of a GPS unit. Male individuals (at least 2-3 individuals) were caught with the help of a sweep net for bioacoustic studies from different points in the areas and they were brought to the room/laboratory alive by taking them into special cages. After the male calling song recordings of the collected tettigoniid individuals, species identifications were made using the related literature, with the help of a LEICA S8 APO model stereo microscope with a digital camera connected imaging feature. After bioacoustic and morphological examinations, tettigoniid specimens were deposited in tubes containing 96% ethyl alcohol, in a deep freezer at -20 °C, in the Zoology Museum (Hakkari) at Hakkari University Biological Diversity Application and Research Centre.

Song recording and bioacoustic analyses

In tettigoniids, the male calling song is produced by rubbing the stridulatory file teeth on the back of the left wing against the inner edge of the other wing (Heller, 1988). The calling songs were recorded either in the field or in the laboratory under room conditions. Song recordings were made with a high-quality condenser microphone (frequency response flat up to 20 kHz) capable of digital sound recording and with the TASCAM-DR 100 device (a recorder using audio technical) that can record from 44.1 kHz to 48 kHz. In this system, the song records of individuals were recorded on the memory card, or the data were transferred by directly connecting to the computer with a USB connection cable. The bioacoustic data transferred to the computer were analysed with the GoldWave and Cool Edit 96 programs as well as the Labview 7 program. On the other hand, Turbolab 4.0 (Stemmer AG) program was used to prepare and print out the oscillograms. All means of bioacoustic characters are given with standard deviation (±).

Bioacoustic terminology

The bioacoustic terminology used in the calling song description of Orthoptera species includes differences according to the taxa studied and researchers (Heller, 1988; Ragge & Reynolds, 1998; Kolics et al, 2008; Şirin et al, 2014) although there were some standardization terminology studies for insect (Baker & Chesmore, 2020). The absence of standardized terminology for the song description of taxa belonging to the Orthoptera order causes some difficulties in definitions (Robinson & Hall, 2002). This is due to the difference in the structural features of the wing movements, which constitute the basic calling song type in the Orthoptera order. For this reason, the terminologies in Heller (1988), Ragge & Reynolds (1998), Taylan (2005), Heller (2006), Sevgili et al, (2011), Şirin et al, (2014), Şirin et al, (2019), Taylan et al, (2019) and Baker & Chesmore (2020) provided for the bioacoustic analysis and comparison of calling songs. Therefore, a synthesis of bioacoustic terminology was used in the study (Fig. 2). *Calling song*, song produced by an isolated male; phrase, a first-order assemblage of syllables; *syllable*, the song produced by one opening-closing movement cycle of the tegmina; *syllable interval*, time from end of last impulse to beginning of first impulse of the next syllable; *pulse*, an indivisible and most basic unit of stridulatory song that generated during the opening or closing movement of the wing.

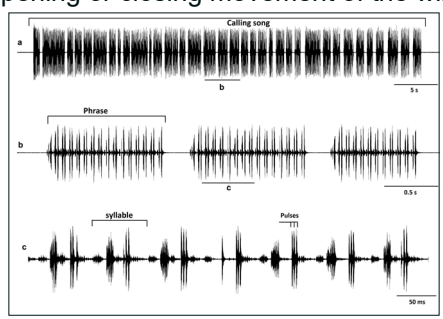

Figure 2. Bioacoustic terminology for studied tettigoniids. *Tettigonia viridissima:* a) calling song, b) three phrases, c) seven syllables.

RESULTS

The bioacoustic analysis of two genera (*Kurdia* and *Novadrymadusa*) documented first time with present study and in total six species (*Bradyporus latipes*, *Isophya hakkarica*, *Kurdia uvarovi*, *Novadrymadusa karabagi*, *Psorodonotus hakkari,* and *Squamiana supericola*) were presented for the first time in this study. However the bioacoustic analysis of seven species (*Conocephalus fuscus*, *Pezodrymadusa indivisa*, *Polysarcus zacharovi*, *Tettigonia armeniaca*, *Tettigonia caudata*, *Tettigonia viridissima* and *Uvarovistia satunini*) were examined the first time from Hakkari populations. Additionally, the bioacoustic analyses of three species (*Apholidoptera kurda*, *Saga hakkarica* and *Uvarovistia zebra*) distributed in Hakkari were re-evaluated with using new individuals.

Among the examined sixteen tettigoniid species, the distribution, localities, song descriptions, and bioacoustic data of six tettigoniid species collected in the field studies in Hakkari province were presented for the first time in this study and also bioacoustic data of *Polysarcus zacharovi* (which has a different song structure compared with previous data) are presented.

Bioacoustics

Subfamily: Tettigoniinae Krauss, 1902

Genus: *Novadrymadusa* Demirsoy, Salman & Sevgili, 2002

Novadrymadusa karabagi Demirsoy, Salman & Sevgili, 2002

Distribution: The *N. karabagi* is an Anatolian endemic species and is distributed in Hakkari and Şırnak provinces (Demirsoy, Salman, & Sevgili, 2002; Ünal, 2018; Taylan et al, 2021).

Song recording: Male individuals of the species were collected from Hakkari Cilo-Sat Mountains National Park, Kırıkdağ, Cennet-Cehennem valley, Sipilhane location, 37°31.538´ N, 43°58.877´ E, 2570 m, 07.08.2021 (legs. M.S. Taylan & C. Avcı), and song recordings were taken from three males at room conditions (26.5 °C) (song recording: M.S. Taylan & C. Avcı).

Song description: Six song recordings of three males were examined. The male calling song consists of phrases consisting of polysyllabic sequences (Fig. 3a). The duration of the polysyllabic series varies between 8-78 s (28 ± 11.25) and includes 10-11 syllables in 100 ms (Fig. 3b). Bioacoustic analyses showed that syllables consist of two parts. The first part begins with a short, low-amplitude pulse-like structure (6-9 ms (8.15 ± 0.85 ms)) and continues with a longer and higher (21-25 ms (22.25 ± 2.11 ms)) amplitude unit. The second part is similar to the last unit of the first part of syllable and its duration varies between 22-27 ms (24.62 ± 2.48 ms). While syllable duration was 78–138 ms (102.15 ± 7.22 ms) (Fig. 3c), it was determined that there was a 22–78 ms (35.41 ± 7.25 ms) interval between syllables.

Genus: *Psorodonotus* Brunner von Wattenwyl, 1861

***Psorodonotus hakkari* Kaya, Korkmaz & Çıplak, 2013**

Distribution: *P. hakkari* is endemic species to Hakkari province (Kaya, Korkmaz, & Çıplak, 2013; Taylan et al, 2021).

Song recording: Male individuals of the species were collected from Hakkari, Berçelan Plateau, 37°42.502´ N, 43°43.907´ E, 2930 m, 04.07.2021 (legs. M.S. Taylan & C. Avcı) and song recordings were taken from three males at room conditions (27°C) (song recording: M.S. Taylan).

Song description: Six song recordings were examined from three males. The male calling song consists of a sequence of regularly repeated syllables (Fig. 4a), with an interval of 320–495 ms (404 ± 28.25 ms) between syllables. The syllable duration varies between 78-102 ms (90.23 ± 7.65 ms) (Fig. 4b). As a result of oscillographic analysis, it was seen that each syllable consisted of 5 elements (Fig. 4c). The first and second elements are short and low amplitude pulses. The third element has a longer and higher amplitude (16-22 ms (19.14 ± 3.11 ms)) and contains 9-12 distinguishable pulses. The fourth elements are also short and of low amplitude, similar to the first and second elements. The last element (5th) is the longest and highest amplitude part, it contains 13-17 visible pulses and its duration varies between 24-31 ms (28 ± 3.72 ms).

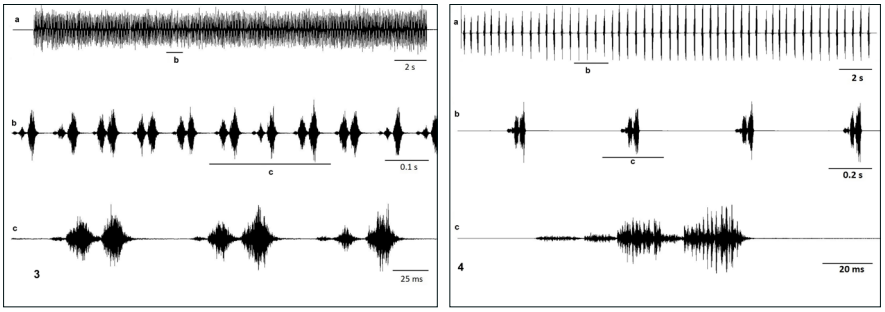

Figure 3-4. 3) Male calling song of *Novadrymadusa karabagi* Demirsoy, Salman & Sevgili, 2002. a) polysyllabic sequence, b) number of syllable in 100 ms, c) three complete syllables; 4) Male calling song of *Psorodonotus hakkari* Kaya, Korkmaz & Çıplak, 2013. a) regularly repeated syllable sequences, b) four complete syllables, c) a complete syllable.

Genus: *Squamiana* Zeuner, 1941

***Squamiana supericola* (Ünal, 2018)**

Distribution: *S. supericola* (Ünal, 2018) is an endemic species to Şırnak and Hakkari provinces (Ünal, 2018; Taylan et al, 2021).

Song recording: Male individuals of the species were collected from Hakkari, Berçelan Plateau, 37°40.380´ N, 43°43.622´ E, 2840 m, 08.VII.2021 (legs. M.S. Taylan & C. Avcı), and song recordings were taken from two males at room conditions (27 °C) (song recording: M.S. Taylan & C. Avcı).

Song description: Four song recordings were examined from two males. The male calling song consists of regularly repeated phrases that including of polysyllabic

sequences (Fig. 5a). The phrase is almost crescendo (Fig. 5b), and the syllables at the beginning of the phrase have low amplitude and continue until 1/2-1/3 of the phrase. Then the phrase continues and ends with high amplitude syllables. The duration of the polysyllabic sequence varies between 2.40-6.27 s (3.26 ± 1.23 s), with a gap of 0.25-1.78 s (0.51 ± 0.22 s). The syllables in the first part phrase cannot be distinguished and contain 8-11 (9.15 ± 0.81) pulses. Syllables in the second half of the phrase can be distinguished and its duration varies between 14-18 ms (16.4 ± 1.48 ms). The number of syllables in 100 ms is 6-8 (7.14 ± 0.65) (Fig. 5c).

Subfamily: Bradyporinae Burmeister, 1838

Genus: *Bradyporus* Charpentier, 1825

***Bradyporus* (*Callimenus*) *latipes* (Stål, 1875)**

Distribution: *B. latipes* (Stål, 1875) is distributed in Eastern Anatolia (Malatya, Bitlis, Kars, Elazığ, Şırnak, and Hakkari) (Ünal, 2011b; Ünal, 2017; Taylan et al, 2021).

Song recording: Male individuals of the species were collected from, Hakkari, Mergabutan Ski Centre, 37°34.877´ N, 43°40.795´ E, 2480 m, 06.08.2021, (legs. M.S. Taylan & C. Avcı) and song recordings were taken from three males at room conditions (27 °C) (song recording: M.S. Taylan & C. Avcı).

Song description: Eight song recordings of three males were examined. The male calling song consists of polysyllabic sequences (Fig. 6a) and the duration of the polysyllabic sequences varies between 6.120–78.540 s (65.320 ± 12.05 s). The syllable duration varies between 58-119 ms (62.15 ± 14.25 ms), and the number of words in 100 ms is 2. Oscillographic analyses show that the syllables are composed of two elements, one which has a high amplitude (42-51 ms ± 2.14 ms), and the other has a low amplitude (19-30 ms ± 3.01 ms) (Fig. 6b, 6c). It is determined that there is a gap of 7–14 ms (10.08 ± 2.45 ms) between syllables.

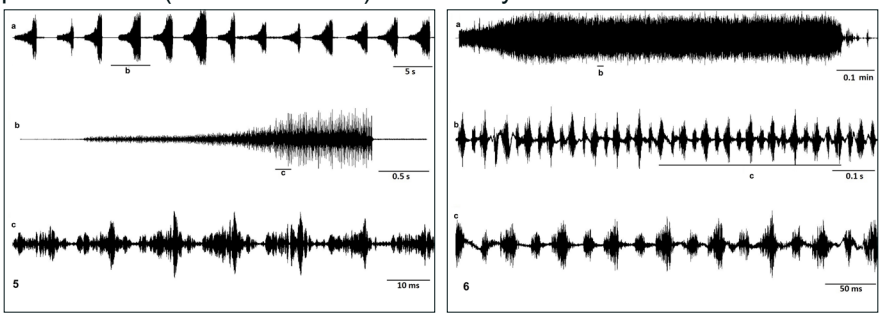

Figure 5-6. 5) Male calling song of *Squamiana supericola* (Ünal, 2018). a) regularly repeated phrases, b) a complete phrase, c) syllable numbers in 100 ms; 6) Male calling song of *Bradyporus* (*Callimenus*) *latipes* (Stål, 1875). a) polysyllabic sequences, b) syllables in 1s, c) eight complete syllables.

Subfamily: Phaneropterinae Burmeister, 1838

Genus: *Isophya* Brunner von Wattenwyl, 1878

***Isophya hakkarica* Karabağ, 1962**

Distribution: The *I. hakkarica* Karabağ, 1962 is a sub-endemic species to Anatolia and show distribution in Hakkari and Iraq (Karabağ, 1962; Taylan et al, 2021).

Song recording: Male individuals of the species were collected from Hakkari, Berçelan Plateau, 37°40.387´ N, 43°43.636´ E, 2835 m, 08.08.2021 (legs. M.S. Taylan & C. Avcı) and song recordings were taken from three males at room conditions (27 °C) (song recording: M.S. Taylan & C. Avcı).

Song description: Six song recordings of three males were examined. The male calling song consists of an irregular phrase consisting of 2 or 3 syllables of irregular duration and its duration varies between 6-12 s (11.15 ± 3.4 s) (Fig. 7a). The phrase usually consists of two different syllables types (Fig. 7a). First syllable is short-high amplitude and the second syllable long-low-amplitude. The short and high amplitude syllable has a duration of 242-302 ms (297.38 ± 29.15 ms) and has a reverse-crescendo pattern (Fig. 7b). The duration of long and low-amplitude syllable varies between 1.224-1.255 s (1.238 ± 0.18 s), and the first 5-7 structural elements can be distinguished (Fig. 7c).

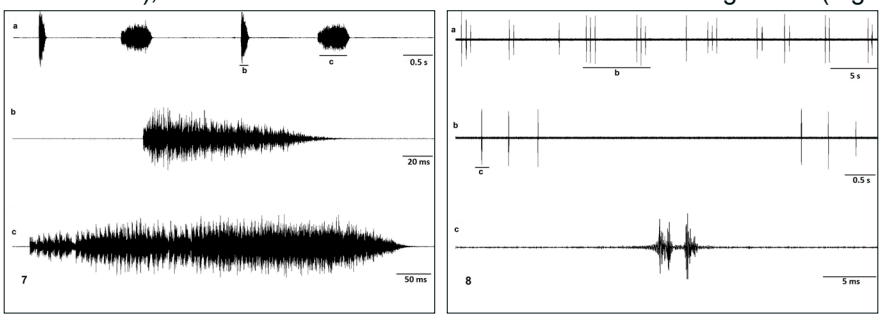

Figure 7-8. 7) Male calling song of *Isophya hakkarica* Karabağ, 1962. a) complete song, b) short and high amplitude syllable, c) long and low amplitude syllable; 8) Male calling song of *Kurdia uvarovi* Karabağ, 1975. a) complete song (variable syllable groups), b) two different triple syllable groups, c) a complete syllable and pulses.

Genus: *Kurdia* Uvarov, 1916

Kurdia uvarovi Karabağ, 1975

Distribution: *K. uvarovi* is an endemic species to Anatolia and distributed only in Hakkari province (Karabağ, 1975; Taylan et al, 2021).

Song recording: Male individuals of the species were collected from Hakkari, Yüksekova-Şemdinli road, Haruna valley, 37°21.685´ N, 44°32.264´ E, 1690 m, 17.06.2002 (leg. H. Sevgili) and song recordings were taken from two males in the field (22 °C) (song recording: H. Sevgili).

Song description: Four song recordings of two males were examined. The male calling song consists of regular 2 or 3 (rarely single) syllable groups (Fig. 8a). There is a gap of 2.240-4.760 s (3.214 ± 0.742 s) between syllable groups and 412-495 ms (460.25 ± 42.40 ms) between syllables (Fig. 8b). Oscillographic analyses show that syllables are composed of 2 or 3 high amplitude short pulses (Fig. 8c). The syllable duration varies between 3-6 ms (4.72 ± 1.06 ms).

Genus: *Polysarcus* Fieber, 1853

***Polysarcus zacharovi* (Stshelkanovtzev, 1910)**

Distribution: The species *P. zacharovi* is distributed in Caucasia, Transcaucasia, and Eastern Anatolia (Karabağ, 1958; Ünal, 2010; Sevgili et al, 2012; Taylan et al, 2021).

Song recording: Male individuals of the species were collected from Hakkari, Berçelan plateau, 37°40.392´ N, 43°43.642´ E, 2840 m, 14.08.2021 (legs. M.S. Taylan & C. Avcı) and song recordings were taken from two males in laboratory conditions (27 °C) (song recording: M.S. Taylan).

Song description: Three song recordings of two males were examined. The song consists of isolated single syllables (Fig. 9a). There is a gap of 0.802-7.75 s (3.915 ± 2.35 s) between syllables (Fig. 9b). Oscillographic analyses show that syllables are usually composed of one element (Fig. 9c). The syllable duration ranged from 64 to 73 ms (70.12 ± 2.42 ms). Rarely, there is a short, low-amplitude pulse at the beginning of the syllable, lasting 4-6 ms (5.25 ± 0.85 ms). This element contains a gap of 5-7 ms with the main song.

The remaining nine species of tettigoniids examined in Hakkari province (*Apholidoptera kurda* (Fig. 10), *Conocephalus* (*Anisoptera*) *fuscus* (Fig. 11), *Pezodrymadusa indivisa* (Fig. 12), *Saga hakkarica* (Fig. 13), *Tettigonia armeniaca* (Fig. 14), *Tettigonia caudata* (Fig. 15), *Tettigonia viridissima* (Fig. 16), *Uvarovistia satunini* (Fig. 17), and *Uvarovistia zebra* (Fig. 18), mainly show similar patterns with previous studies (Heller, 1988; Heller, 2006; Grzywacz, Heller, Warchałowska-Śliwa, Karamysheva, & Chobanov, 2017; Şirin et al, 2014, 2019; Taylan et al, 2019). For these reason, song descriptions of these species were not presented again. The comparisons of song descriptions of these nine species with previous studies' song data are given in the next section.

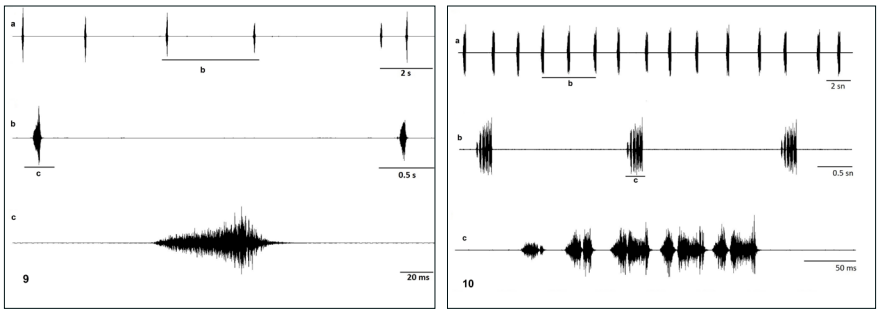

Figure 9-10. 9) Male calling song of *Polysarcus zacharovi* (Stshelkanovtzev, 1910). a) complete song (repeated syllables), b) two complete syllables, c) a complete syllable; 10) Male calling song of *Apholidoptera kurda* (Uvarov, 1916). a) complete song (regularly repated phrases), b) three complete phrases, c) a complete phrase and its syllables and elements

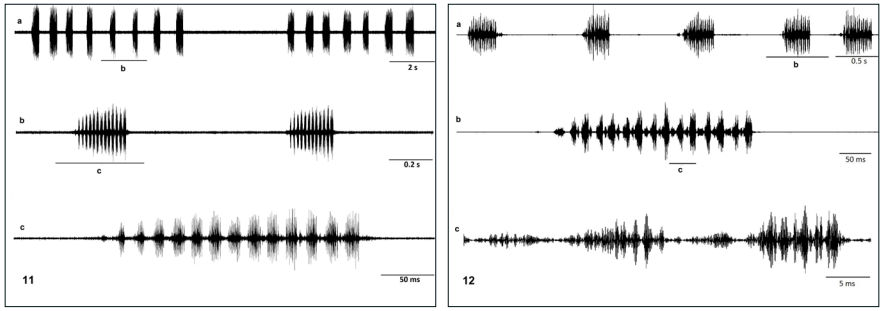

Figure 11-12. 11) Male calling song of *Conocephalus* (*Anisoptera*) *fuscus* (Fabricius, 1793). a) syllable sequences, b) two complete syllables, c) a complete syllable sequence; 12) Male calling song of *Pezodrymadusa indivisa* Karabağ, 1961. a) phrase sequences, b) a complete phrase, c) two complete syllables.

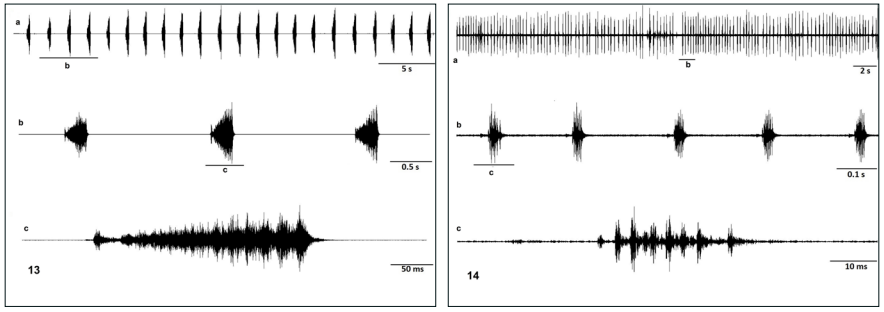

Figure 13-14. 13) Male calling song of *Saga hakkarica* Sirin & Taylan, 2019. a) complete song (regularly repeated phrases), b) three complete phrases, c) a complete phrase and syllables; 14) Male calling song of *Tettigonia armeniaca* Tarbinsky, 1940. a) repeated syllable series, b) five complete syllables, c) a complete syllable.

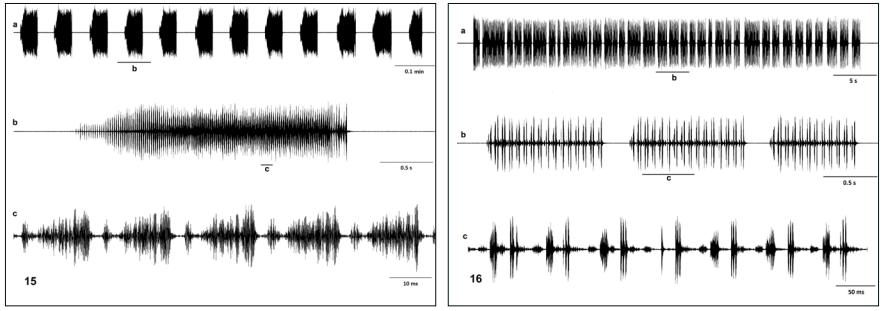

Figure 15-16. 15) Male calling song of *Tettigonia caudata* (Charpentier, 1845). a) polysyllabic sequences, b) a complete plysyllabic sequence, c) number of syllables in 100 ms; 16) Male calling song of *Tettigonia viridissima* (Linnaeus, 1758). a) calling song (polysyllabic phrase sequences), b) three complete phrases, c) seven complete syllables.

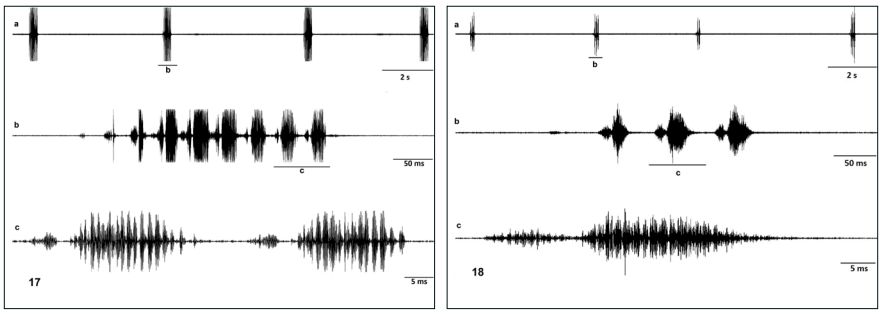

Figure 17-18. 17) Male calling song of *Uvarovistia satunini* (Uvarov, 1916). a) repeated syllables groups, b) a complete syllable group (octal), c) two complete syllables; 18) Male calling song of *Uvarovistia zebra* (Uvarov, 1916). a) repeated syllables groups, b) a complete syllable group (octal), c) a complete syllable and elements.

CONCLUSIONS AND DISCUSSION

In this paper, the bioacoustic evaluation of the male calling songs of sixteen species of Tettigoniidae family belonging to five subfamilies (ten species of Tettigoniinae, three species of Phaneropterinae and one species of each of Bradyporinae, Conocephalinae and Saginae subfamilies) distributed in Hakkari province was carried out. Among the sixteen tettigoniid species distributed in Hakkari, six of them are Anatolian endemic (*Saga hakkarica*, *Psorodonotus hakkari*, *Novadrymadusa karabagi*, *Pezodrymadusa indivisa*, *Squamiana supericola*, and *Kurdia uvarovi*), seven are sub-endemic or have restricted distribution to Anatolia (*Apholidoptera kurda*, *Bradyporus* (*Callimenus*) *latipes*, *Isophya hakkarica*, *Polysarcus zacharovi*, *Tettigonia armeniaca*, *Uvarovistia zebra* and *Uvarovistia satunini*) and three (*Conocephalus* (*Anisoptera*) *fuscus*, *Tettigonia caudata*, and *Tettigonia viridissima*) have a relative widespread distribution (Taylan et al, 2019; Sirin et al, 2019; Taylan et al, 2021).

Bioacoustic data of six species (*Bradyporus* (*Callimenus*) *latipes*, *Isophya hakkarica*, *Kurdia uvarovi*, *Novadrymadusa karabagi*, *Psorodonotus hakkari*, and *Squamiana supericola*) were presented for the first time in this study. Additionally, eight species (*Conocephalus fuscus*, *Pezodrymadusa indivisa*, *Polysarcus zacharovi*, *Tettigonia armeniaca*, *Tettigonia caudata*, *Tettigonia viridissima* and *Uvarovistia satunini*) were examined the first time from Hakkari province. On the other hand, male calling song analyses of three other tettigoniid species (*Apholidoptera kurda*, *Saga hakkarica*, and *Uvarovistia zebra*) distributed in Hakkari were re-evaluated in this study.

The genus *Bradyporus* Charpentier, 1825, is represented by 11 species in Anatolia (Ünal, 2011a; Cigliano et al, 2022). Among these species, song descriptions of *B*. (*Callimenus*) *avanos* Ünal, 2011, *B*. (*Callimenus*) *dilalatus* (Stål, 1875), *B*. (*Bradyporus*) *dasypus* (Illiger, 1800), *B*. (*Callimenus*) *toros* Ünal, 2011, and *B*. (*Callimenus*) *conophallus* Ünal, 2011 were given in previous studies (Heller, 1988; Şirin et al, 2014; Taylan et al, 2019). However, interspecies bioacoustic comparisons only have been examined by Taylan et al, (2019) and they noted that *B*. *avanos*, *B. toros*, and

B. conophallus have species-specific calling song characters, with differences in syllable period. According to bioacoustic analysis of *B. latipes*, it was determined that the species produced a simple calling song, similar to other species belonging to the genus *Bradyporus* in general song structure but differed from other species in terms of syllable duration and number of syllables/100 ms. *B. latipes* has a longer calling song structure than other species in terms of syllable duration. In species *B. latipes*, the syllable duration varies between 58-119 ms, while the number of syllables/100 ms is 2. When these values are compared with *B. avanos* (syllable duration: 21-28, syllables number in 100 ms: 4), *B. toros* (syllable duration: 16-19, syllable number in 100 ms: 6-7), and *B. conophallus* species (syllable duration: 11-16, syllables number in 100 ms: 7-8), we identified that *B. latipes* produces species-specific songs.

The genus *Isophya* Brunner von Wattenwyl, 1878, is represented by 45 species/subspecies in Turkey (Sevgili, 2004; Ünal, 2011a; Cigliano et al, 2022). Sevgili (2004), divided the genus *Isophya* into six species groups in a morphological and bioacoustics-based comprehensive study of the Anatolian members of the genus. Sevgili (2004), performed a bioacoustic analysis of *I. hakkarica*, (a species which was examined in the present work), and placed it together with *I. schneideri* Brunner von Wattenwyl, 1878, *I. acrita* (as sp.n) and *I. acuminata* Brunner von Wattenwyl, 1878, species in the *schneideri*-subgroup of the *rectipennis*-species group. However, Ünal (2010) examined *I. hakkarica*, together with *I. schneideri*, *I. sikorai* Ramme, 1951, *I. karabaghi* Uvarov, 1940, *I. cania* Karabag, 1975 and *I. thracica* Karabag, 1962, species within the *schneideri*-species group in a morphological-based study. On the other hand, *I. iraca* Maran, 1977, which is geographically closest to *I. hakkarica*, was noted as a synonym of *I. hakkarica* in Taylan et al, (2021). Zhantiev, Korsunovskaya & Benediktov, (2017) examined some *Isophya* species in Eastern Europe, the Caucasus, and surrounding countries and in their bioacoustics-based study, the male calling song description of *I. schneideri* from the *schneideri*-species group was presented. When the bioacoustics of *I. hakkarica* is compared with *I. schneideri*, it is seen that both species have similar song pattern and the calling song of both species have simple calling song structure consisting of two syllables of different duration and type. However, while the short and high amplitude first syllable duration in *I. hakkarica* ranged between 242 and 302 ms (297.38 ± 29.15 ms), this duration was 239.3 ms in *I. schneideri* species. The second syllable, which is long and has low amplitude, has duration of 1.224-1.255 s (1.238 ± 0.18 s) in *I. hakkarica*, while ranged between 600-1100 ms in *I. schneideri*. Therefore, although the general song pattern of these two species is similar, it has been determined that the syllables differ in terms of duration.

The genus *Kurdia* Uvarov, 1916, belongs to the Phaneropterinae subfamily and Barbitistini tribe and is represented by two species in the world. Among these species, *Kurdia nesterovi* Uvarov, 1916, is very restricted to North Iraq, while *Kurdia uvarovi* Karabağ, 1975 is endemic to Hakkari province in Anatolia (Karabağ, 1975; Taylan et al, 2021). Since the male calling song of the *Kurdia nesterovi* was not defined, the male calling song of the *Kurdia uvarovi* could not be compared within the genus. However, the male calling song of *Kurdia uvarovi* consists of regularly spaced double or triple

(rarely single) isolated syllable groups. On the other hand, the song structure of this species shows similarity to some species of the *Leptophyes* Fieber, 1853 (especially *L. sicula*) and *Isophya* (especially *I. brunneri* Retowski, 1888) genera in the tribe Barbitistini (Kleukers, Odé, & Fontana, 2010; Zhantiev et al, 2017).

Novadrymadusa Demirsoy, Salman & Sevgili, 2002, genus belongs to the subfamily Tettigoniinae and tribe of Drymadusini, and is distributed only in Iran and Anatolia (Ramme, 1939; Demirsoy et al, 2002). One of the species belonging to the genus, *Novadrymadusa kurda* (Uvarov, 1930), has not yet been studied bioacoustically. The other species of the genus, *Novadrymadusa karabagi* is endemic to Hakkari and Şırnak provinces in Anatolia. Since the other species of the genus could not be examined bioacoustically, an intra-genus evaluation could not be presented here. However, bioacoustic analyses show that *Novadrymadusa karabagi* has a simple calling song structure and the male calling song consists of phrases consisting of the polysyllabic sequences. It has been determined that the duration of the polysyllabic sequence varies between 8-78 s (28 ± 11.25 s) and 10-11 syllables are produced in 100 ms. Furthermore, it is structurally similar to *Lithodusa helverseni* Heller, 2009, which calling song structure and descriptions are known in the same tribe (Heller, & Korsunovskaya, 2009).

The genus *Psorodonotus* Brunner von Wattenwyl, 1861 is known with 13 species from Anatolia and 10 of these species are endemic or sub-endemic to Anatolia (Ünal, 2011a; Taylan et al, 2019; Cigliano et al, 2022). In previous studies, it has been noted that species within the genus (*P. caucasicus* (Fischer von Waldheim, 1846), *P. suphani* Taylan & Şirin, 2014, *P. davisi* Karabag, 1957, *P. ebneri* Karabag, 1952, and *P. rugulosus* Karabag, 1952) have species-specific calling song structures (Şirin et al, 2014, Taylan et al, 2019). The male calling song of the *P. hakkari* Kaya, Korkmaz & Çıplak, 2013, consists of regularly spaced syllables, with an interval of 320–495 ms (404 ± 28.25 ms) between syllables. The syllable duration varies between 78-102 ms (90.23 ± 7.65 ms). As a result of oscillographic analysis, it was seen that each syllable consisted of five elements (short + short + high + short + high) and with this structure, it is similar to the *P. davisi*. However, it is also distinguished from this species and other species in the genus in terms of duration (Şirin et al, 2014; Taylan et al, 2019).

There are nine species belonging to the genus *Squamiana* Zeuner, 1941, and among these species, the calling song description of *S. kurmana* (Ramme, 1951) has been obtained by Taylan et al, (2019). The calling song of the *S. supericola* (Ünal, 2018) species was given in this study and differences were determined in terms of structure and duration with *S. kurmana* species (Taylan et al, 2019). In *S. supericola*, the male calling song is in a crescendo structure, consisting of polysyllabic sequences, pulses in syllables cannot be distinguished, and the number of syllables in 100 ms was determined as 6-8 (7.14 ± 0.65). On the other hand, in *S. kurmana*, the male calling song consists of regular phrases. The syllable structure consists of two distinguishable parts and the calling song contains only one syllable in 100 ms (Taylan et al, 2019). In this context, it is observed that the male calling song has a simple song structure and is species-specific within the genus.

Furthermore, although calling song descriptions were noted before, the male calling song of the *Conocephalus* (*Anisoptera*) *fuscus*, *Pezodrymadusa indivisa*, *Polysarcus zacharovi*, *Tettigonia armeniaca, Tettigonia caudata*, *Tettigonia viridissima* and *Uvarovistia satunini* species, which were recorded for the first time in Hakkari province, were examined oscillographically and it was observed that Hakkari populations of these species (except *Polysarcus zacharovi* species) were similar to the populations given from other provinces in terms of calling song structure and characters of male calling song.

The genus *Polysarcus* Fieber, 1853, generally produces a complex song and it was previously given from the *P. zigana* Ünal & Chobanov, 2013, and the Erzurum population of the *P. zacharovi* species (Heller, 1988; Korsunovskaya, 2008; Taylan et al, 2019). However, in our study, distinct bioacoustic differences were detected between the Hakkari population of *P. zacharovi* and other populations. Hakkari population of *P. zacharovi* species does not produce complex calling song but produces simple song structures. The male calling song in this population consists of isolated syllables, and oscillographic analyses show that a syllable is usually composed of one element. On the other hand, when the male calling song recording of Jafari et al, (2015), the *P. denticauda* (Charpentier, 1825) species from Iran is examined, it is seen that this song is not complex, but consists of polysyllabic sequences. Therefore, the male call song of the Iranian population of this species is more similar to the song structure of the Hakkari population of the *P. zacharovi*. Taylan et al, (2021) noted some morphological differences between Hakkari population and the type of P*. zacharovi*. To clarify the taxonomic status of the Hakkari population, a phylogenetic evaluation is required in which the Erzurum population of *P. zacharovi*, Iran population of *P. denticauda* species and Giresun population of *P. zigana* species will be considered together. However, it should not be ignored that (i) the difference in the male calling song of the Hakkari population of *P. zacharovi* may also be due to the inability to record the complete calling song, (ii) or the signal given in the article may be a signal of protest, which is also emitted by katydids of the genus *Polysarcus* (Korsunovskaya, 2008: Zhantiev & Korsunovskaya, 2015) or (iii) this population belongs to a new species. However, it was observed that all three calling songs recorded from single males at different times had a simple song structure.

Additionally, when the current bioacoustic data of *Apholidoptera kurda*, *Saga hakkarica*, and *Uvarovistia zebra* species whose Hakkari population male calling songs were re-evaluated, it was discovered that they were similar to the original song description (Şirin et al, 2019; Taylan et al, 2019). Otherwise, a shorter phrase duration (253 ms) was noted in the phrase duration of the current population of *Saga hakkarica*.

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CONFLICT OF INTEREST DISCLOSURE

There is no conflict of interest.

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Aenictus dirangensis sp. nov. (Hymenoptera: Formicidae), a New Species of *Aenictus ceylonicus* Group from India

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ABSTRACT

A new species of the *Aenictus ceylonicus* group, *Aenictus dirangensis* sp. nov., is described and illustrated based on the worker caste. The new species occurs in North-Eastern Himalayas and shows morphological similarities with *A. yangi* Liu, Hita Garcia, Peng, & Economo (2015) and *A. wilaiae* Jaitrong & Yamane (2013). *Aenictus dirangensis* sp. nov. can be separated from both by shape of the subpetiolar process and body sculpture. The new species also resembles *A. khaoyaiensis* Jaitrong & Yamane (2013) but clearly differentiated in having 2-6 toothed mandible/denticles between subapical and basal teeth. An identification key to the Asian *Aenictus ceylonicus* group is also updated.

Keywords: Dorylinae, key, taxonomy and army ants.

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INTRODUCTION

The army ant genus *Aenictus* is the largest genus belonging to the subfamily Dorylinae. It is represented by 199 species and 25 subspecies from the World (Bolton, 2022). The genus is widely distributed in the Old World tropics and sub tropics. Southeast Asia is the home to majority of species, while Afrotropics also served as a significant centre of diversity for the genus. Additionally, a small number of species can be found in southern Palearctic zone and there are several species known to exist in Australia (Boroweic, 2016).

Shuckard (1840) named *Aenictus* after a male from India because of its "enigmatical structure." The status of the genus was disputed as many taxonomists considered it as a genus in the subfamily Dorylinae (Mayr, 1865; Dalla Torre, 1893; Emery, 1895; Borgmeier, 1954) whereas, it was mostly considered the only genus of the subfamily Aenictinae by others (Bolton, 1990, 1995; Baroni Urbani, Bolton, & Ward, 1992; Wu & Wang, 1995; Bolton, 2003; Jaitrong & Yamane, 2011, 2013). Brady, Fisher, Schultz, & Ward, (2014) revised the status of the genus and placed it in the subfamily Dorylinae based on molecular data.

The entire contemporary taxonomy of the genus is based on the worker caste even though the type species is only known from the male. A taxonomic revision of the species of the ant genus *Aenictus* present in the Indo-Australian region was published by Wilson (1964), including the description of new taxa and identification keys to the species. It was followed by the subsequent studies on the genus from different parts of the World: Terayama & Yamane (1989) from Japan; Terayama & Kubota (1993), Jaitrong & Nabhitabhata (2005), Jaitrong (2015), Khachonpisitsak, Yamane, Sriwichai, & Jaitrong, (2020) from Thailand; Zhou & Chen (1999), Zhou (2001) from Guangxi; Jaitrong & Nur-Zati (2010) from Malay Penninsula; Jaitrong, Yamane, & Chanthalangsy, (2011) from Laos; Jaitrong, Yamane, & Wiwatwitaya (2010), Jaitrong, Yamane, & Tasen (2012), Jaitrong & Yamane (2011, 2012), Wiwatwitaya & Jaitrong (2011), Jaitrong & Hashimoto (2012), Jaitrong & Wiwatwitaya (2013) from Oriental region and Southeast Asia; Zettel & Sorger (2010) from Borneo and the Philippines; Gomez (2022) from Afrotropical region.

Jaitrong & Yamane (2011) identified 12 species groups of the ant genus *Aenictus* found throughout the eastern portion of the Oriental area, as well as the Indo-Australian and Australasian regions. Out of these species groups, the *A. ceylonicus* species group is the most diverse and is represented by 29 species from Asia (Jaitrong & Yamane, 2011, 2013; Staab, 2015; Liu, Garcia, Peng, & Economo, 2015, Antony & Prasad, 2022). The *A. ceylonicus* group is distinguished from the other species groupings by the following characteristics: linear mandibles, a gap between the mandibles and anterior border of the clypeus when mandibles are closed, and the anterior clypeal margin is almost straight or feebly concave, with no denticles (Jaitrong & Yamane, 2011, 2013).

The characteristics that distinguish army ants are the result of a set of evolutionarily linked physiological, behavioral, and anatomical traits, collectively referred to as the army ant adaptive syndrome. This syndrome includes the construction of temporary

bivouac nests and a nomadic lifestyle (Kronauer, 2009). They are prominent for their large-scale predation activities, known as “raids,” in which a large number of ants forage concurrently over a large region, collecting a variety of prey, primarily attacking other ants, social wasps and termites, including nearly all kinds of arthropods (Santschi, 1933; Schneirla, 1971; Rościszewski & Maschwitz, 1994; Gotwald, 1976, 1995; Hirosawa et al, 1998). Therefore, army ants have been labelled as keystone species that have a significant impact on the diversity and composition of terrestrial ecosystems (Franks & Bossert, 1983; Perez-Espona, 2021).

In India the genus is represented by 33 species and 2 subspecies (Bingham, 1903; Bharti et al, 2012, 2016; Antony & Prasad, 2022). In this present study, we document a new species of ant genus *Aenictus* belonging to the *A. ceylonicus* species group from India based on the worker caste. An identification key to the Asian *A. ceylonicus* group based on workers has been updated from Jaitrong & Yamane (2013), Liu et al, (2015) and Staab (2015) and complemented with digital images.

MATERIALS AND METHODS

Specimens were studied using a Nikon SMZ 1500 stereo zoom microscope with a maximum magnification of 112.5X. Digital images of the specimens were prepared using a MP (Micro 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 millimeters with an ocular micrometer fitted on a Nikon SMZ 1500 stereomicroscope.

Images of the species from Jaitrong & Yamane (2013); Liu et al, (2015) and Staab (2015) or provided by http://www.antweb.org/ were compared for determining new species identity. Morphological terminology and standard measurements follow Gomez (2022).

HL: In full face view, head Length measured from the clypeal distal border to the occipital line, measured in the vertical symmetry axis; clypeal teeth and other structures are left out of the measurements.

HW: maximum Head Width. Usually the head mid-length, but in some cases it can be lined up with the mandibular insertions.

SL: Scape Length, excluding the basal constriction and the condylar bulb.

WL: Weber’s Length, in lateral view from the pronotal declivity to the inferopropodeal lobe.

PH: Petiole Height, in lateral view, from the base of the petiolar sclerite to maximum dome height.

PL: Petiole Length, in lateral view, from the rearmost point of the sclerite, to the anteriormost point of the anterololateral petiolar ridge.

PPH: Postpetiole Height, in lateral view, from the base of the postpetiolar sclerite to maximum dome height.

PPL: Postpetiole Length, in lateral view, from the rearmost point of the sclerite, to theanteriormost postpetiolar point.

CS = (HL+HW)/2. Represents the cephalic size independently from cephalic shape.

CI = HW/HL*100. Measures head elongation. Indexes greater than 100 indicate oblong heads.

CSR = Csmax/Csmin*100. Cephalic Size Range. Measures the relative difference in size from maxima to minima workers, thus, the size variability for a given species.

PI = PL/PH*100. Petiolar Index, with higher index corresponding to more elongate petiole.

PPI = PPL/PPH. PostPetiolar Index, with higher index corresponding to more elongate postpetiole.

SIW = SL/HW*100. Relative length of the scape in its classical definition.

SIL = SL/HL*100. Length of scape relative to head length. The almost complete absence of clypeus in this genus makes this index preferable to SIW as it translates immediately into the percentage of head reached by the scape when laid back.

Depositories

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

RESULTS

Aenictus dirangensis sp. nov. (Figs: 1-4)

Types: Holotype worker (PUAC—T 07), from India, Arunachal Pradesh, Dirang, 27.3566º N, 92.23720º E, 1560m, handpicking, 03.ix.2019, Tarun Dhadwal leg. Paratypes: Fourteen workers (PUAC-T 08-17), same data as holotype.

Worker Measurements

Holotype: HL 0.54; HW 0.46; WL 0.78; SL 0.36; PL 0.24; PH 0.19; PPI 0.20; PPH 0.18; GL 0.80; SI 78.26; CI 85.18; CSR; SIW 16.56; SIL 66.66; PPI 90.00; PI 79.16. **Paratypes** (n=10): HL 0.52-0.56; HW 0.42-0.48; WL 0.76-0.82; SL 0.36-0.40; PL 0.22-0.26; PH 0.19-0.21; PPI 0.18-0.20; PPH 0.18-0.19; GL 0.78-0.82; SI 85.71-83.33; CI 80.76-85.71; CSR 109.58; SIW 85.71-83.33; SIL 69.23-71.42; PPI 95.00-100.00; PI 80.76-86.36.

Description: Head in full-face view longer than broad, with convex lateral sides and weakly concave posterior margin. Antennal scape short, reaching mid-length of the head. Frontal carina comparatively long, extending beyond posterior margin of torulus. Parafrontal ridge absent. Anterior clypeal margin feebly concave medially; median portion of the margin meets the lateral portion forming blunt angle on each side. Masticatory margin, with a broad apical tooth, followed by medium-sized subapical tooth, 4 denticles, and smaller basal tooth; basal margin nearly straight. Maximum width of gap between anterior clypeal margin and mandibles 0.8 and 1.0 times broader than

maximum width of mandible. Promesonotum in lateral view, convex and eventually slopes into distinct metanotal groove. Mesopleuron in lateral view moderately long, smooth median strip separates it from metapleuron. The metapleural gland bulla large, its maximum diameter is twice the distance between propodeal spiracle and metapleural gland bulla.

Propodeum in lateral view, with convex dorsal outline, converging more sharply posterad; propodeal corner angular, with distinct tooth. Propodeal declivity sinuous in lateral view. Petiole node as long as high with dorsal margin convex, subpetiolar process well developed and subrectangular with acute anterior corners and blunt posterior corners. Postpetiole shorter than petiole with convex dorsal outline.

Head, mandible and scape completely smooth and polished; mesopleuron, metapleuron, and lateral face of propodeum reticulate; promesonotum smooth and shiny; dorsal face of propodeum reticulate; with some transverse striations. Petiole and postpetiole dorsum smooth with lateral faces moderately reticulated.

Head, mesosoma dorsally with relatively sparse standing hairs mixed with sparse shorter hairs and legs with long sparse hairs. Head including antennal scape reddish-brown; mandible, mesosoma dark reddish-brown; petiole, postpetiole, gaster and legs yellowish-brown.

Remarks: *Aenictus dirangensis* sp. nov. shows similarities with *Aenictus yangi* Liu, Hita Garcia, Peng, & Economo (2015) and *A. wilaiae* Jaitrong & Yamane (2013) and *A. khaoyaiensis* Jaitrong & Yamane (2013).

It can be differentiated from *A, yangi* on the basis of following characteristics: 1) the subpetiolar process in *Aenictus yangi* is elongate, subrectangular, and slightly projecting anteroventrally (well developed and subrectangular with anterior and posterior corners acutely or bluntly angular in *A. dirangensis* sp. nov.); 2) in *Aenictus yangi* the dorsal face of the propodeum is mostly smooth and shiny and the lateral face is partly smooth and shiny whereas (dorsal face of the propodeum has transverse striation and the lateral face is reticulated in *A. dirangensis* sp. nov.); 3) in *A. yangi* lateral propodeal margins gently sloping posteriorly (lateral propodeal margins converge more sharply posteriorly in *A. dirangensis* sp. nov.); 4) the relative width of the propodeal face seems narrower in *A. yangi* (the relative width of the propodeal face wider in *A. dirangensis* sp. nov.); 5) postpetiole angular in lateral view in *A. yangi* (postpetiole convex in profile view in *A. dirangensis* sp. nov.); 6) the metanotal groove is weakly impressed in *A. yangi* (distinct in *A. dirangensis* sp. nov.).

From *A. wilaiae* it can be differentiated based on following characteristics: 1) in A. *wilaiae* promesonotal dorsum smooth and shiny except for anteriormost portion punctate (promesonotal dorsum entirely smooth and shiny in *A. dirangensis* sp. nov.); 2) in *A. wilaiae* subpetiolar process generally very low, with its anteroventral corner angulate and ventral margin convex (well developed and subrectangular with acute anterior corners and blunt posterior corners in *A. dirangensis* sp. nov.); 3) in *A. wilaiae* mesopleuron with longitudinal rugae, lateral face of propodeum with 2-3 short longitudinal rugae, petiole and postpetiole densely punctate (mesopleuron,

lateral face of propodeum reticulate, petiole and postpetiole dorsum smooth with lateral faces moderately reticulated).

However from *A. khaoyaiensis* it can be differentiated based on the following characteristics: 1) in *A. khaoyaiensis* Mandible with 0-1 tooth/denticle between subapical and basal teeth (mandible with 3-4 teeth/denticles) (Mandible with 2-6 teeth/denticles between subapical and basal teeth (mandible with more than 4 teeth/denticles) in *A. dirangensis* sp. nov.); 2) subpetiolar process in *A. khaoyaiensis* is low, with its anteroventral corner angulate and ventral margin weakly convex (well developed and subrectangular with acute anterior corners and blunt posterior corners in *A. dirangensis* sp. nov.); 3) in *A. khaoyaiensis* promesonotum smooth except for anteriormost portion punctate and mesopleuron with several irregular longitudinal rugae (promesonotum smooth and mesopleuron reticulated in *A. dirangensis* sp. nov.); 4) mandibles striate in *A. khaoyaiensis* (in *A. dirangensis* sp. nov. mandibles smooth).

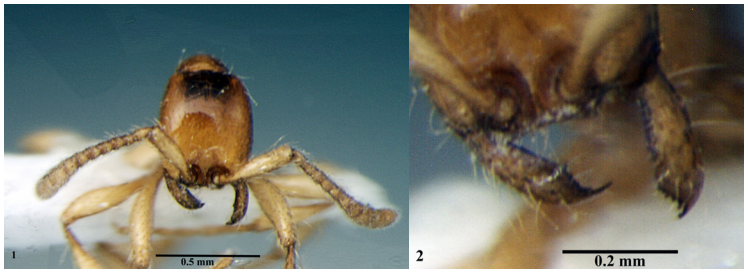

Figure 1-2. *Aenictus dirangensis* sp. nov. 1) head in full face view, 2) mandibles.

Figure 3-4. *Aenictus dirangensis* sp. nov. 3) body in profile view, 4) body in dorsal view.

Habitat: The workers were manually collected from beneath a stone in Dirang village falling in West Kameng district of Arunachal Pradesh. The village is situated at an elevation of 1560 meters, with an average daily temperature of 20˚C. The ground is covered with grass and surrounded by Kiwi plantation.

Etymology: The species has been named after the type locality.

Identification key to the *A. ceylonicus* group

Key to Asian *A. ceylonicus* group species based on worker caste, modified after Jaitrong & Yamane's key (2013) with inputs of Liu et al, (2015) and Staab (2015).

1. Mandible with 2-6 teeth/denticles between subapical and basal teeth (mandible with more than 4 teeth/denticles) (Fig. 5a)……..... *2*

- Mandible with 0-1 tooth/denticle between subapical and basal teeth (mandible with 3-4 teeth/denticles) (Fig. 5b)..... 22

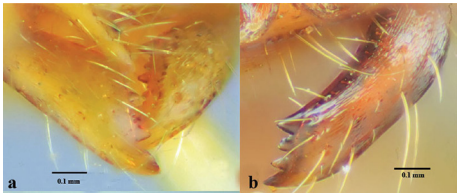

Figure 5. Mandibles of *A. ceylonicus* group species in full face view. a) *A. lifuiae*, b) *A. maneerati* (Images are from Jaitrong & Yamane, 2013).

2. Promesonotum entirely sculptured (punctate or reticulate)..... 3

- Promesonotum largely smooth and shiny..... 4

3. Promesonotum sparsely reticulate or superficially micropunctate; antennal scape superficially shagreened; petiole cylindrical, clearly longer than high (Fig. 6a)..... .....*A. cylindripetiolus* Jaitrong & Yamane

- Promesonotum densely punctate; antennal scape micropunctate; petiole round or subangular, almost as long as high (Fig. 6b)..... *A. thailandianus* Terayama & Kubota

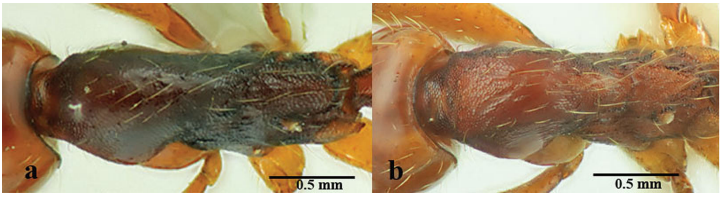

Figure 6. Promesonotum. a) *A. cylinderipetiolus*, b) *A. thailandianus*. (Images are from Jaitrong & Yamane, 2013).

4. Subpetiolar process prominent 5

- Subpetiolar process not prominent, weakly developed 8

5. Dorsum of mesonotum and petiole finely reticulate.....6

- Dorsum of mesonotum and petiole smooth and shiny.....7

6. Subpetiolar process rectangular-trapezoidal, its ventral outline with a thin almost transparent lamellae (Fig. 7a); masticatory margin of mandible with 4 (rarely 3) denticles (total number of mandibular teeth 6-7, including apical, subapical, and basal tooth) .....*A. hoelldobleri Staab*

- Subpetiolar process rectangular, its apex very acute and directed downwards medially (Fig. 7b); masticatory margin of mandible with 6 denticles (total number of mandibular teeth 9 including apical, subapical, and basal tooth).....*A. wudangshanensis* Wang

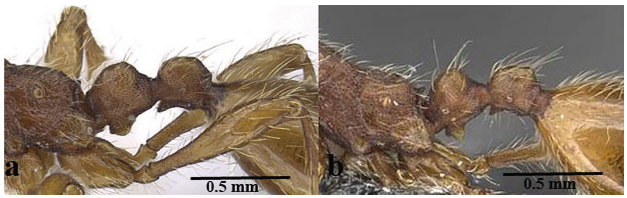

Figure 7. Subpetiolar process. a) *A. hoelldobleri* (CASENT0914932), b) *A. Wudangshanensis* (CASENT0914927).

7. Metanotal groove distinct; lateral propodeal margins converge more sharply posterad; pospetiole convex in profile; subpetiolar process subrectangular with acute anterior corners and blunt posterior corners (Fig. 8a, b) ……..*A. dirangensis* sp. nov.

- Metanotal groove weakly impressed; lateral propodeal margins gently sloping posteriorly; pospetiole angular in profile; subpetiolar process relatively elongated, and slightly projecting anteroventrally (Fig. 8c, d)..... ..... *A. yangi* Liu, Hita Garcia, Peng & Economo

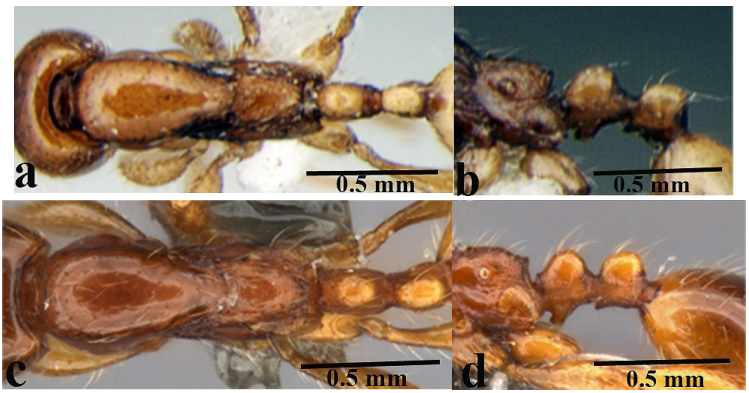

Figure 8. Dorsal view and subpetiolar process. a-b) *A. dirangensis* sp. nov., c-d) *A. yangi* (Liu et al, 2015).

8. Dorsal face of propodeum mostly smooth and shiny, the lateral face of propodeum partly smooth and shiny; postpetiole entirely smooth and shiny (Fig. 9a)……..…9

- Propodeum entirely sculptured; postpetiole entirely sculptured or with a smooth and shiny small area on the dorsal face (Fig. 9b)…….....12

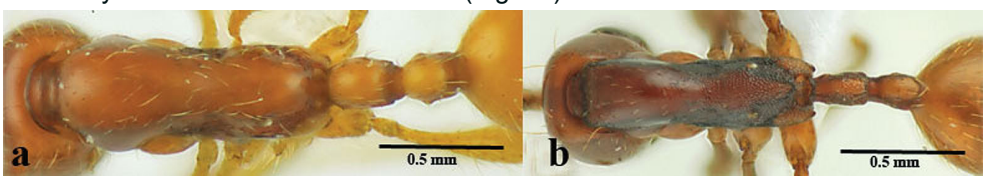

Figure 9. a) Dorsal view of *A. longicephalus*, b) Dorsal view of *A. appressipilosus*. (Images are from Jaitrong and Yamane, 2013)

9. The declivity of propodeum with lateral carinae, but not demarcated basally by a transverse carina (Fig. 10a)……..……..*A. longicephalus* Jaitrong & Yamane

- The declivity of propodeum is shallowly concave, encircled with a rim (Fig. 10b).....10

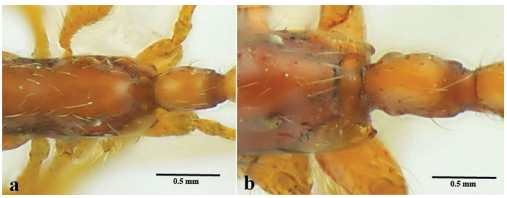

Figure 10. Showing propodeal declivity. a) *A. longicephalus*, b) *A. baliensis*. (Images are from Jaitrong and Yamane, 2013),

10. Distal 2/3 of basal margin of mandible straight, proximal 1/3 narrowed toward the base of mandible; anterior clypeal margin straight; petiole smaller than postpetiole (Fig. 11a, b) ..... *A. minipetiolus* Jaitrong & Yamane

- Basal margin of mandible feebly concave; anterior clypeal margin concave; petiole larger than or as large as postpetiole (Fig. 11c, d) …….....11

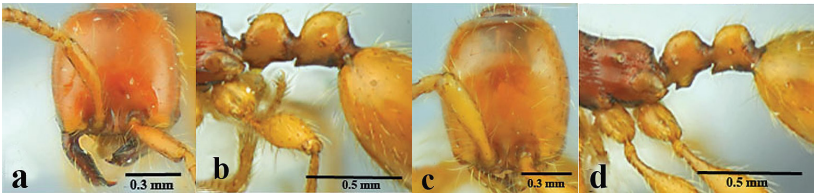

Figure 11. Showing head, petiole and postpetiole. a-b) *A. minipetiolus*, c-d) *A. baliensis* (Images are from Jaitrong and Yamane, 2013).

11. Promesonotum is strongly convex and sloping gradually to the metanotal groove; subpetiolar process with angular posteroventral corner (Fig. 12a)…… ……..... .....……..... .....*A. baliensis* Jaitrong & Yamane

- Mesosoma almost flat dorsally or feebly convex; subpetiolar process lower, with its posteroventral corner rounded (Fig.12b).....*A. wiwatwitayai* Jaitrong and Yamane

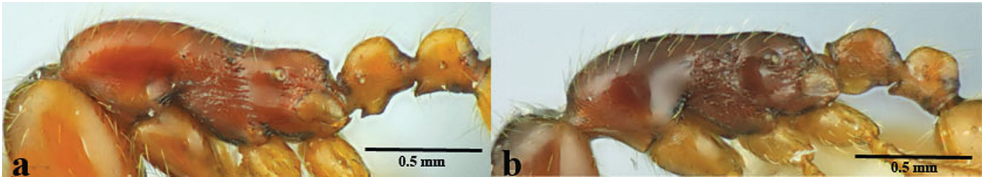

Figure 12. Profile view. a) *A. baliensis*, b) *A. wiwatwitayai*. (Images are from Jaitrong and Yamane, 2013).

12. Vertex with sparse standing hairs and with a few short appressed hairs; promesonotum with few appressed hairs and a few decumbent hairs (Fig. 13a).....13

- Vertex and promesonotum with dense standing or decumbent hairs (Fig. 13b)…....14

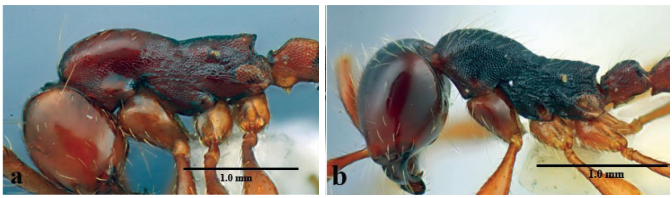

Figure 13. Profile view. a) *A. appressipilosus*, b) *A. pinkaewi*. (Images are from Jaitrong and Yamane, 2013).

13. Vertex with two long-standing hairs mixed with a few short appressed hairs..... .....*A. appressipilosus* Jaitrong & Yamane

- Vertex with only few short appressed hairs.....*A. malakkaparensis* Antony & Prasad

14. Occipital corner modified into a small lobe in profile and dorsal view (Fig. 14a).....*A. gonioccipus* Jaitrong & Yamane

- Occipital corner without modifications in profile and dorsal view (Fig. 14b).....15

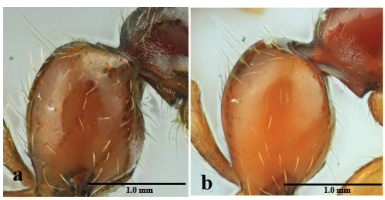

Figure 14. Head profile view. a) *A. gonioccipus*, b) *A. lifuiae* (Images are from Jaitrong and Yamane, 2013).

15. Mesonotum straight in dorsally slope down backward; posterodorsal corners of propodeum protruding and dentate, declivity concave..... *A. henanensis* Li & Wang

- Mesonotum weakly or strongly convex in dorsal outline; posterodorsal corners of propodeum angular nearly right-angled, declivity shallowly concave.....16

16. Masticatory margin of mandible with large acute apical tooth followed by a series of 6-7 denticles of two sizes, the larger alternating with 1-2 smaller; the gap between anterior clypeal margin and mandibles relatively small or indistinct, with maximum width shorter than the maximum width of the mandible (Fig. 15a)..... .....*A. lifuiae* Terayama

- Masticatory margin of mandible with large acute apical tooth followed by a medium-sized subapical tooth, 2-5 denticles, and a medium-sized basal tooth; the gap between anterior clypeal margin and mandibles relatively large and distinct, with maximum width longer than the maximum width of the mandible (Fig. 15b).....17

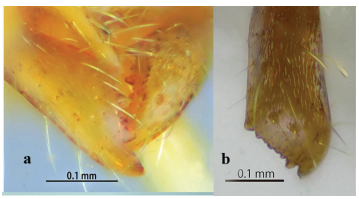

Figure 15. Mandibles. a) *A. lifuiae*, b) *A. thailandianus*. (Images are from Jaitrong and Yamane, 2013).

17. Subpetiolar process subtriangular, its apex directed backward, anteroventrally not angular; postpetiole elevated posteriorly; its posterior face much steeper than anterior face (Sumatra) (Fig. 16a).....*A. itoi* Jaitrong & Yamane

- Subpetiolar process subrectangular, its apex directed forward , anteroventrally angular; postpetiole with roundly convex dorsal outline (Fig.16b, c).....18

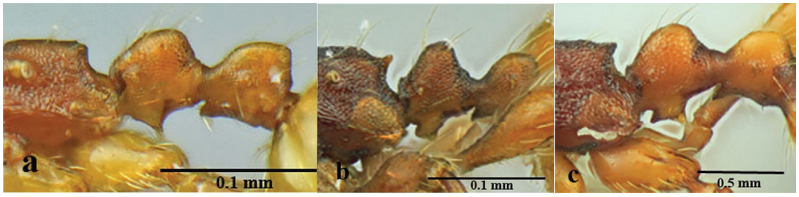

Figure 16. Subpetiolar process. a) *A. itoi*, b-c) *A. gonioccipus* and *A. jawadwipa*. (Images are from Jaitrong and Yamane, 2013).

18. Subpetiolar process rectangular, ventrally with anterior and posterior corners.....19

- Subpetiolar process low, its ventral outline convex, almost straight or feebly concave, and anteroventral corner acutely angular……..…..….....….....21

19. Dorsal outline of propodeum weakly convex; metapleural gland bulla weakly sculptured (Vietnam) .....*A. eguchii* Jaitrong & Yamane

- Dorsal outline of propodeum straight; metapleural gland bulla strongly sculptured (puncto-reticulate) ……..…….....……20

20. Posteroventral corner of subpetiolar process angular (not spiniform).....
.....*A. kodungallurensis* Antony & Prasad

- Posteroventral corner of subpetiolar process acutely produced ventrally (spiniform)
.....*A. jawadwipa* Jaitrong & Yamane

21. Promesonotum in profile weakly convex; propodeal dorsum feebly convex; petiole sessile, its posterior face encircled with a thin carina; postpetiole almost as long as petiole (Fig. 17a).....*A. wilaiae* Jaitrong & Yamane

- Promesonotum in profile strongly convex and forming a dome; propodeal dorsum clearly straight; petiole subsessile, its posterior face not encircled with a carina; postpetiole slightly shorter than petiole (Philippines) (Fig. 17b).....
.....*A. pilosus* Jaitrong & Yamane

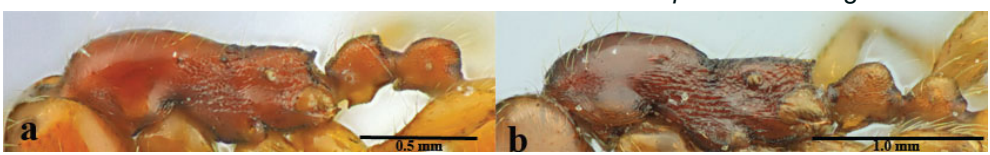

Figure 17. Profile view. a) *A. wilaiae*, b) *A. pilosus*. (Images are from Jaitrong and Yamane, 2013).

22. Mandible with 3 teeth including apical and basal teeth (Fig. 18a). 23
Mandible with 4 teeth including apical and basal teeth (Fig. 18b)…....24

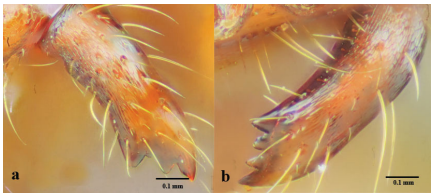

Figure 18. Mandible. a) *A. watanasiti*, b) *A. maneerati*. (Images are from Jaitrong and Yamane, 2013).

23. Occipital corner rounded in lateral view; promesonotum in profile strongly convex and forming a dome; petiole almost as long as high, with high node, and almost as long as postpetiole (Fig. 19a)……..*A. watanasiti* Jaitrong & Yamane

- Occipital corner shallowly rounded in lateral view; promesonotum in profile with weakly convex or almost flat dorsal outline; petiole clearly longer than high, with low node, and slightly longer than postpetiole Fig. 19b).....*A. concavus* Jaitrong & Yamane

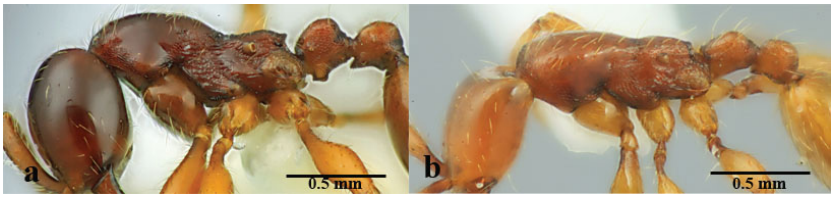

Figure 19. Profile view. a) *A. watanasiti*, b) *A. concavus***.** (Images are from Jaitrong and Yamane, 2013).

24. Dorsal and lateral faces of pronotum largely smooth and shiny; petiole almost as long as high; head slightly longer than or almost as long as broad (CI 90- 100) (Fig. 20a).....25

- Dorsal and lateral faces of pronotum entirely sculptured (superficially puncto-reticulate, punctate to reticulate); petiole cylindrical, distinctly longer than high; head slightly shorter than broad (CI 102-112) (Fig. 20b).....27

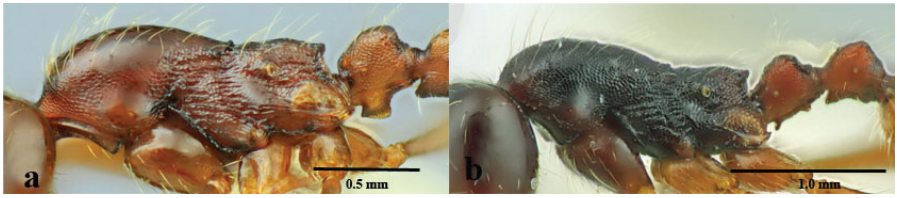

Figure 20. Profile view. a) *A. formosensis*, b) *A. maneerati.* (Images are from Jaitrong and Yamane, 2013).

25. Anterior clypeal margin distinctly concave; metanotal groove distinct, deep; foretibia relatively short, its length less than 0.5 times of head width; mesopleuron finely punctate without longitudinal rugulae (Fig. 21a)……..*A. brevipodus* Jaitrong &Yamane

- Anterior clypeal margin almost straight or feebly concave; metanotal groove indistinct; foretibia relatively long, its length about 0.9-1.0 times as long as head width; mesopleuron with longitudinal rugulae (Fig. 21b).....26

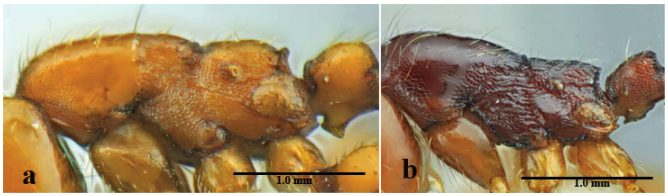

Figure 21. Mesosoma. a) *A. brevipodus*, b) *A. khaoyaiensis*. (Images are from Jaitrong and Yamane, 2013).

26. Subpetiolar process low, ventral outline weakly convex, its anteroventral corner angular (Thailand) (Fig. 22a).....*A. khaoyaiensis* Jaitrong & Yamane

- Subpetiolar process well-developed, subrectangular with a convex ventral lamella, with anterior corners acute and posterior corners bluntly angular (Fig. 22b)…..
.....*A. formosensis* Forel

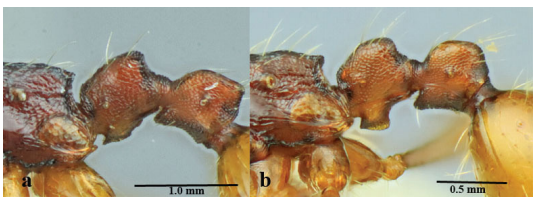

Figure 22. Subpetiolar process. a) *A. khaoyaiensis*, b) *A. formosensis*. (Images are from Jaitrong and Yamane, 2013).

27. Subpetiolar process very low, with anterior and posterior denticles that protrude anteroventrally; head in full-face view rectangular, its posterior margin feebly concave (Thailand) (Fig. 23a, b)…….....*A. maneerati* Jaitrong & Yamane

- Subpetiolar process subrectangular or low, with its anteroventral corner acutely angular and ventral outline straight or convex; head in full-face view subrectangular or rounded, its posterior margin weakly concave, almost straight or weakly convex (Fig. 23c, d).....28

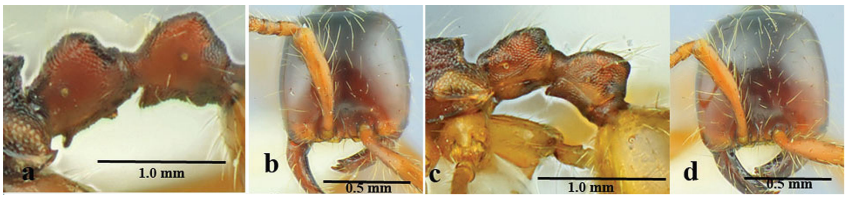

Figure 23. Subpetiolar process and head in full face view. a-b) *A. maneerati* and c-d). *A. fuchuanensis*. (Images are from Jaitrong and Yamane, 2013)

28. Pronotum with central area superficially shagreened or rather smooth and shining, and with lateral face reticulate and shiny; subpetiolar process low, with its anteroventral corner acutely angular and ventral outline concave (Fig. 24a)…….....
.....*A. sundalandensis* Jaitrong &Yamane

- Pronotum micropunctate or reticulate and opaque (at most weakly shining);

subpetiolar process always low, with its anteroventral corner acutely angular and ventral outline almost straight or convex (Fig. 24b)…….....29

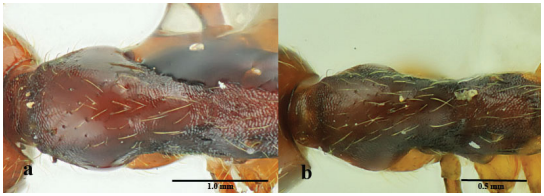

Figure 24. Dorsal view. a) *A. sundalandensis*, b) *A. fuchuanensis.* (Images are from Jaitrong and Yamane, 2013).

29. Pronotum entirely strongly punctate (N. Thailand) (Fig. 25a) ....*A. pinkaewi* Jaitrong & Yamane

- Anterior portion of the pronotum densely punctate, the lateral face of the pronotum finely reticulate (China, Hong Kong, Laos and Thailand) (Fig. 25b)..... *A. fuchuanensis* Zhou

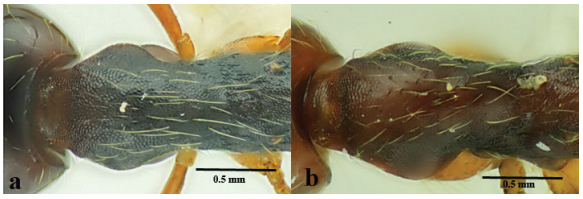

Figure 25. Dorsal view. a) *A. pinkaewi*, b) *A. fuchuanensis.* (Images are from Jaitrong and Yamane, 2013).

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Taxonomic Study of the Tribe Onitini Laporte, 1840 (Coleoptera: Scarabaeidae: Scarabaeinae) from Northern Pakistan

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ABSTRACT

We provide occurrence records of the members of dung beetle's tribe Onitini from the northern parts of Pakistan, including Gilgit-Baltistan, Khyber Pakhtunkhwa Province and Islamabad Capital Territory. This study is based on extensive field sampling from different ecological biomes including Alpine Meadows, the Sub-Alpine Zone, Mountain Temperate Forest, and Subtropical Deciduous Forest. As a result, we report six species under two genera of tribe Onitini: *Onitis falcatus*, *O. lama*, *O. philemon, O. subopacus, O. virens,* and *Cheironitis arrowi*. The genus *Cheironitis* is recorded for the first time from northern parts of Pakistan. We provide photographs of the dorsal and ventral habitus, diagnosis, distribution maps and identification keys to known genera and species in the tribe Onitini from Pakistan.

Keywords: *Onitis*, new record, *Cheironitis*, Scarabaeioidea, dung beetles, Oriental region.

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INTRODUCTION

The tribe Onitini, which is part of the subfamily Scarabaeinae within the family Scarabaeidae, encompasses around 210 species of tunneling beetles belonging to 18 genera that feed solely on feces. The two genera *Cheironitis* and *Onitis* have a relatively high number of species, with *Cheironitis* contains 159 species and *Onitis* having 24 species globally (Krajcik, 2006). Species in these two genera are not only found in Africa, but also have a range that extends into the Palaearctic and Oriental regions. The remaining 16 genera are species poor, consisting of only three species in the Palaearctic (belonging to the genus Bubas) and 15 African endemics (Scholtz, Davis, & Kryger, 2009). The tribe is recognizable by its antennae, which have nine segments, as well as a visible scutellum and pronotum having a pair of noticeable small impressions in middle of the base. The first in-depth study of Onitini in India was conducted by Arrow (1931), where author reported 13 species of *Onitis* and one species of *Cheironitis*. In his monograph, Balthasar (1963b) conducted an investigation of 15 species of *Cheironitis*, 30 species of *Onitis*, and 2 species of *Bubas* from both the Palaearctic and Oriental regions.

Despite their immense diversity, the Scarabaeidae fauna of northern Pakistan has not been investigated due to lack of proper accessibility and research facilities. There are a series of non-coprophagous scarab beetle publications from Pakistan (Abdullah & Roohi, 1968, 1969) and some well-documented taxonomic work on the Oriental dung beetle fauna carried out by Arrow (1931) and Balthasar (1963a, b, c). More than 70 species of Scarabaeinae from Pakistan are listed in Löbl & Smetana (2006)'s "Catalogue of Palaearctic Coleoptera", but not even a single species has been documented from northern Pakistan. However, in the recent decades, new additions to the scarabid fauna of Pakistan has been updated in a number of faunistic, biodiversity, distribution, and ecological studies (Ratcliffe & Ahmed, 2010; Ahmed, Zorn, & Khatri, 2014; Siddiqui, Ahmed, & Khatri, 2014); but unfortunately, the information on northern areas, particularly Gilgit-Baltistan is scarcely sampled or poorly recorded in published literature (Abbas, Bai, & Yang, 2015).

There are at least 18 pronounced climatic regions in Pakistan (Khan, Hassan, & Khan, 2010). The present study areas in the northern parts of Pakistan have diverse macro- and micro-climate zones: the Alpine Zone (alpine meadows, sub-alpine scrub and birch forests, and alpine dry steppe), the Montane Temperate Forest (sub-tropical pine forests, Himalayan moist temperate forests, Himalayan dry temperate forests), and Subtropical Deciduous Forest.

To fill the long-due wallacean gap, this study was carried out to find out the occurrences records of the dung beetles from the Gilgit-Baltistan, Khyber Pakhtunkhwa Province and Islamabad Capital Territory. Besides, we also provide all the historic records in literature of the species in tribe Onitini from Punjab and Sindh Provinces and Azad Kashmir. We provide identification keys, distribution maps, and diagnosis for each species collected from all possible localities to add to our knowledge of the faunal composition of scarabs found in Pakistan.

MATERIAL AND METHODS

Besides the specimens housed at Pakistan Museum of Natural History, specimens belonging to tribe Onitini available in different worldwide institutes were examined (abbreviations are as shown in the text):

CABI - Centre for Agricultural Bioscience International Rawalpindi, Pakistan.

IZAS - Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

MNHN - Muséum National d'Histoire Naturelle, Paris, France.

NHML - Natural History Museum, London, UK.

NIM - National Insect Museum, Islamabad, Pakistan.

ISNB - Belgium, Brussels, Institute Royal des Sciences Naturelles de Belgique.

BMNH - British Natural History Museum.

NMPC - National Museum (Natural History), Department of Entomology, Prague, Czech Republic.

PMNH - Pakistan Museum of Natural History, Islamabad, Pakistan.

ZIN - Russian Academy of Sciences, Zoological Institute, St. Petersburg, Russia.

ZMUC - University of Copenhagen, Zoological Museum, København [= Copenhagen], Denmark.

Sampling Areas

The present study was conducted in three broader habitats, viz., the Alpine Zone, Montane Temperate Forest, and Subtropical Deciduous Forest, in northern Pakistan. For a comprehensive investigation, these regions were further divided into sub-regions. (Fig. 1).

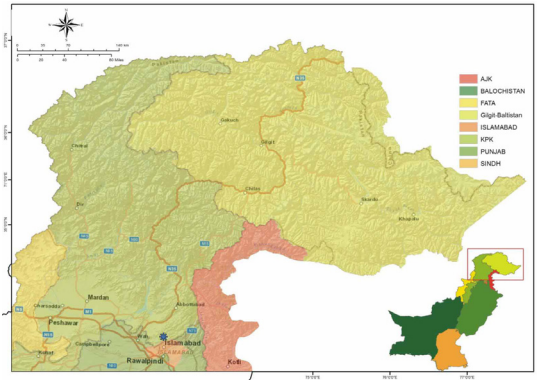

Figure 1. General area sampled for beetle collection for study, Northern Pakistan.

Alpine Zone

The Alpine Zone was typified by continuous grass fields dotted all over with tumbled boulders. The Alpine Zones was subdivided into Alpine meadows, Sub-Alpine scrub and Birch forest, and Alpine dry steppe.

Alpine Meadows

Alpine meadows occurred in the Northern Hazara District, Chitral, Swat, Kohistan, and all regions where mountains extend above the coniferous forest tree line.

Sub-alpine Scrub and Birch Forest

This area consists of upper slopes throughout the higher mountain range of the Himalayas, including the north-eastern corner of Hazara District, Swat, and Kohistan.

Alpine Dry steppe

This is typified by the side valleys of lower Chitral, Kohistan, the western border of Waziristan, and some parts of Safed Koh, Malakand, Swat, and Dir.

Montane Temperate Forest

Montane temperate forests, which are the only real “tall tree” forests in Pakistan, include the following subregions:

Subtropical Pine Forest

This narrow zone ranging from 3000ft to 6500ft is found in the lower Kaghan Valley around Kuwai, Batrassi Pass (Hazara), and lower Swat around Marghazar and Bunair.

Himalayan Moist Temperate Forest

This is predominantly a coniferous forest that gets high rainfall during the monsoon season and has glades of mixed deciduous and broad-leaved species. Parts of eastern Swat, Kohistan, lower Kaghan, Shogran, and Murree Hills are included in this subregion.

Himalayan Dry Temperate Forest

This region consists of the inner and northern ranges of the Himalayas, which are confined to the more sheltered lower slopes, including Jabba Valley in Swat, Dir, Chitral, and the inner valleys of Hazara and Kohistan.

Subtropical Deciduous Forest

Rawalpindi foot-hills, Margalla Hills, Kahota, Lethrar, and Noor Pur Shahan collectively form the subtropical deciduous forest.

From all regions, specimens were collected by hand-picking and using light traps. The collected specimens were stored in vials containing a 70% ethanol solution. All specimens were transferred to the insect repository of the Pakistan Museum of Natural History (PMNH) in Islamabad for systematic study. Specimens were properly prepared and catalogued. Identification were done at the Pakistan Museum of Natural History (PMNH), Islamabad with the help of available literature and determined materials from the IZAS, MNHN, NHML, ZMUC, ZIN, NMPC, PMNH, CABI, and NIM.

The taxonomic characters of the specimens were examined using a Kyowa Optical microscope (Model SDZ-P) Japan. Distributional maps were generated using ArcGIS 10.2 using data from materials examined and information in previous literature from Pakistan.

Taxonomy

Family: Scarabaeidae Latreille, 1802

Subfamily: Scarabaeinae Latreille, 1802

Tribe: Onitini Laporte, 1840

Key to genera of tribe Onitini from Northern Pakistan

1. Scutellum small; base of pronotum not margined (Fig. 5a); protarsi absent; prosternum without any process in both sexes; parameres equal in length to phallobase ..... *Onitis* Fabricius

- Scutellum rather large; base of pronotum margined (Fig. 7a); protarsi present in females, absent in males; in males, prosternum forming a strong bifid process behind procoxae in middle while this process is absent in females; parameres twice as long as phallobase ……..*Cheironitis* van Lansberge

Genus *Onitis* Fabricius, 1798

Onitis Fabricius, 1798: 2. Type species: *Scarabaeus inuus* Fabricius, 1781.

This study recorded five species of *Onitis* from northern areas of Pakistan.

Key to species of genus *Onitis* Fabricius, 1798 from northern Pakistan

1. Pronotum lightly punctured (Fig. 2a) ……..…….…… 2

- Pronotum clearly and deeply punctured (Fig. 5a) ……..…..... 3

2. Pronotum feebly punctuate, punctures shallow and vague (Fig. 2a); pygidium smooth, glabrous; frontoclypeal carina interrupted or with tubercle at middle. ....…..... *O. falcatus* (Wulfen)

- Pronotum lightly punctured, Broad front tibia armed with four external teeth ……..…. *O. lama* van Lansberge

3. Metasternum flat, not grooved ……..……...……...…….. 4

- Metasternum longitudinally grooved in front, ventral surface entirely metallic, upper side rather shining ……..…..…….... *O. philemon* Fabricius

4. Frontoclypeal carina broadly interrupted; frontalcarina with a wide medial gap, base of pronotum not bordered between foveae; protibia with terminal external tooth projecting in front ……..……..……..…….....*O. subopacus* Arrow

- Frontoclypeal carina narrowly interrupted; frontalcarina with only a very short medial gap; protibia with terminal external tooth tapering in front...*O. virens* Lansberge

***Onitis falcatus* (Wulfen, 1786) (Figs. 2a-c)**

Scarabaeus falcatus Wulfen, 1786: 14. Type locality: India.

Onitis himaleyicus Redtenbacher, 1844: 518. Type locality: India (Kashmir: Himalaya).

Onitis kiuchii Masumoto, 1996: 88. Type locality: Thailand.

Diagnosis. The posterior part of the head is irregularly granular. Clypeus is oval moderately strongly and lightly rugulose. Clypeo-frontal carina is widely interrupted in the middle, and there is a short frontal tubercle behind it and in front a short oblique clypeal carina. The base of pronotum is strongly rounded but not distinctly lobed. The pronotum is lightly and rather sparingly punctured without a well-marked median groove or line. The pygidium is thick, unpunctured, and smooth. The elytra are finely striate, with 1, 3, and 5th intervals a little raised and distinctly narrower than the 2nd and 4th, and the meta-sternum sides are densely hairy and also punctured densely.

Material examined. PAKISTAN. Khyber Pakhtunkhwa: Abbottabad, 14.08.2010, 3♂♂, 1♀; Besham, 25.08.2012, 3♂♂, 2♀♀; Buner, 09.09.2007, 2♂♂, 3♀♀; Swat, 12.07.2012, 1♂; Malakand, 06.08.2013, 3♂♂, 3♀♀; Mansehra, 12.07.2008, 4♂♂, 1♀, leg. M. Abbas.

Distribution. Pakistan. Azad Kashmir: Rawalakot; Khyber Pakhtunkhwa: Dera Ismail Khan; Sindh: Tharparkar (Siddiqui, Ahmed, & Khatri, 2014); Bangladesh, Bhutan, Cambodia, India, Myanmar, China, Philippines, Laos, Malaysia, Vietnam and Thailand (Hanboonsong & Masumoto, 2000; Bai, Yang, & Zhang, 2006; Sewak, 2009; Gupta, Chandra, & Khan, 2017; Cheung et al, 2018; Han, Choi, & Park, 2021).

Remarks. Previously recorded by Arrow (1931) from Pakistan. The species is widely distributed in the Himalayan region and semi-arid zones but so far has not recorded from the trans-Himalaya and coastal areas. This forest-dwelling species is attracted to light and feeds on fresh and old cattle dung, mainly of buffaloes and cows, and is rarely found in accumulated dung.

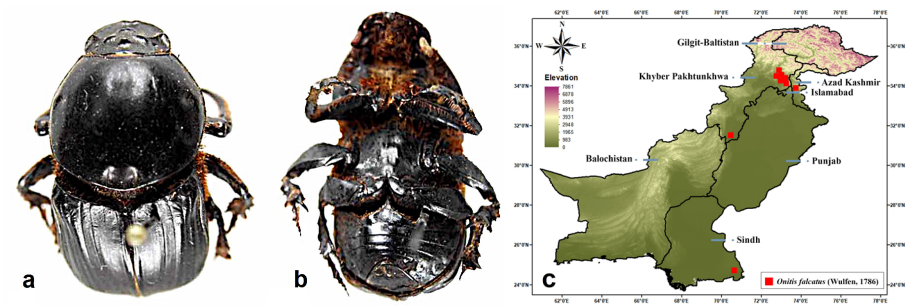

Figure 2. *Onitis falcatus* (Wulfen). a) dorsal, b) ventral habitus, c) regional distribution map in Pakistan.

Onitis lama Lansberge, 1875 (Figs. 3a-c)

Onitis lama Lansberge, 1875: 123. Type locality: India (Himalayas).

Diagnosis. The head is strongly elevated, with a slightly acute frontal carina, with a short straight transverse carina, and a trisinuate vertex carina. Front angles of prothorax blunt sides strongly rounded, base almost completely rounded. Front femur in males have a sharp oblique tooth beyond middle on lower edge and hind femur with a tooth near end of lower edge. Broad front tibia armed with four external teeth.

Material examined. PAKISTAN. Khyber Pakhtunkhwa: Abbottabad, 02.07.2013, 1♂, 3♀♀; Buner, 18.07.2006, 5♀♀; Peshawar, 20.05.2008, 4♂♂; Swat, 14.08.2012, 4♂♂, 1♀; Islamabad: 12.06.2009, 3♂♂, 4♀♀, leg. M. Abbas.

Distribution. Pakistan. Khyber Pakhtunkhwa: Swat (Kanju) (Siddiqui et al, 2014); India (Arrow, 1931; Sewak, 2009; Gupta et al, 2017).

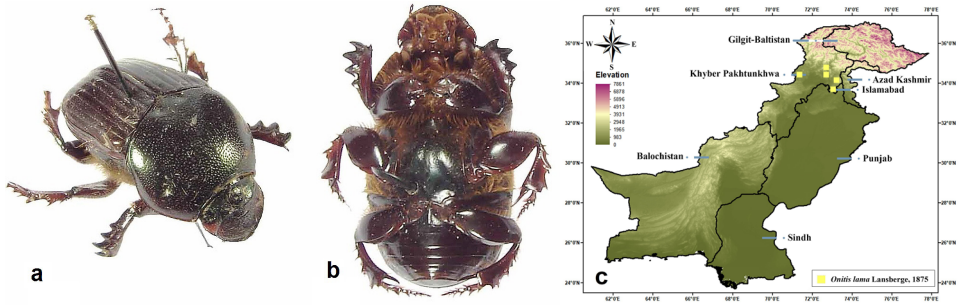

Figure 3. *Onitis lama* (Lansberge). a) dorsal, b) ventral habitus, c) regional distribution map in Pakistan.

Onitis philemon Fabricius, 1801 (Figs. 4a-c)

Onitis philemon Fabricus, 1801: 30. Type locality: India.

Onitis distinctus Lansberge, 1875: 138. Type locality: India.

Diagnosis. Head is rugulose with the ocular lobes. Irregularly punctured pronotum and without clearly marked median lines, in front the lateral margins are straight and in middle strongly rounded. The pygidium is very feebly and sparsely punctured, and the elytra are moderately strongly striate and sparsely punctured. The clypeus is granulate in males, and closely transversely rugose in females. The middle femur has a sharp tooth near the end of the lower edge. Hind trochanter is a little toothed beneath (Figs 4a-b).

Material examined. PAKISTAN. Khyber Pakhtunkhwa: Abbottabad, 01.07.2012, 2♂♂, 4♀♀; Chitral, 20.07.2012, 5♂♂, 4♀♀; Kohat, 21.08.2007, 2♂♂, 5♀♀; Nowshera, 18.08.2013, 4♂♂, 5♀♀; Mansehra, 01.07.2006, 3♂♂, 4♀♀; Peshawar, 11.06.2010, 2♂♂, 3♀♀, leg. M. Abbas. **CHINA.** Fujian: Xiamen, 5-7.06.1980, coll. XIA Shi-Yang, 1♀; Hainan: Ya County, 15-25.04.1982, coll. LIU Zhi-Jin, LI Xue-Feng, 2♀♀; Xinglong, 06.02.1981, coll. LIU Zhi-Jin, LI Xue-Feng, 1♀. **VIETNAM.** Hoa-Binh, date unknown, coll. Cooman, 3♂♂, 1♀; Unknown, 1♂, 2♀♀ (Type) [ZMUC]; Unknown, E. Candeze Coll. 3♀♀. (Type of *Onitis distinctus*) [ISNB]. **INDIA.** Bangalore, S. India, 1♂, 06.03.1903; Cap Comorin, Indes Orientales, R. P. Castats, ?.?.1888, 6♂♂, 1♀.

Distribution. Pakistan. Azad Kashmir: Poonch (Rawalakot); Punjab: Toba Tek Singh (Kamalia) (Siddiqui et al, 2014); China, Bengal and India (Bai et al, 2006; Sewak, 2009; Gupta, Chandra, & Khan, 2014, 2017).

Remarks. Widely distributed species found in loam to heavy loam, pastures with high rainfall regions. It is the most abundant and uniformly distributed species of the genus in the forests. It is attracted towards light and feeds on carnivore and herbivore, dung of human, cow and horse. Active throughout the year, prefers fresh or old dung and is more common in dung heaps accumulated for manure and collected along with *O. subopacus*.

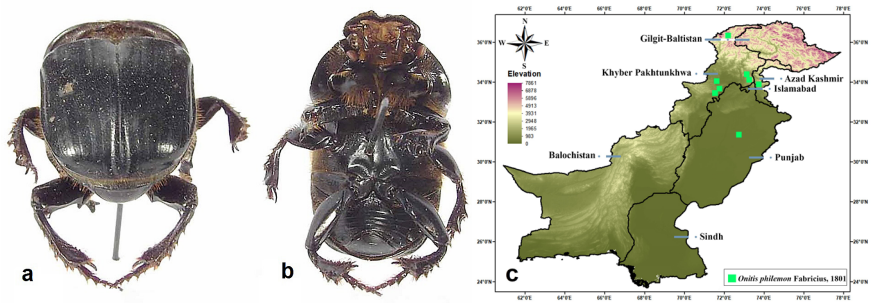

Figure 4. *Onitis philemon* Fabricius. a) dorsal, b) ventral habitus, c) regional distribution map in Pakistan.

Onitis subopacus Arrow, 1931 (Figs. 5a-c)

Onitis philemon Lansberge, 1875: 133. Type locality: Sri Lanka. Junior homonym of *Onitis philemon* Fabricius, 1801.

Onitis subopacus Arrow, 1931: 395. Type locality: Sri Lanka (former name Ceylon). New name for *Onitis philemon* Lansberge, 1875 preoccupied).

Diagnosis. Prothorax and head are reasonably shining and relatively dull elytra. A short transverse clypeal carina is located in front of the pronotum and interrupted carina is somewhat closely but feebly punctured. The pygidium is opaque and un-punctured. The elytra are exceptionally striate, and the 1, 3, and 5th intervals are slightly convex. Middle femur bears a rounded lobe near the middle of the posterior edge and a sharp tooth towards the end, and the middle tibia is slender at the base and strongly and abruptly dilated. Trochanter of the hind leg is sharply toothed (Figs. 5a-b).

Material examined. PAKISTAN. Khyber Pakhtunkhwa: Abbottabad, 11.05.2005, 2♂♂, 5♀♀; Besham, 14.07.2008, 2♂♂, 1♀; Chitral, 21.07.2007, 3♀♀; Dir, 13.04.2014, 2♂♂, 3♀♀; Kohat, 03.07.2013, 4♂♂; Nowshera, 03.07.2010, 7♂♂, 5♀♀; Peshawar, 23.08.2012, 4♂♂, 5♀♀; Swat, 10.06.2012, 2♂♂; Punjab: Murree, 13.06.2006, 1♂, 5♀♀; Islamabad: 02.5.2009, 5♀♀, leg. M. Abbas.

Distribution. Pakistan. Azad Kashmir: Poonch (Rawalakot), Sudhanoti (Palandri); Gilgit-Baltistan: Gilgit (Naltar); Khyber Pakhtunkhwa: Abbottabad, Swat (Marghazar); Punjab: Toba Tek Singh (Kamalia); Sindh: Malir (Siddiqui et al, 2014); India, Myanmar, Thailand, Malaysia, China, Sri Lanka, Afghanistan, Indonesia, Cambodia, Vietnam, Nepal (Hanboonsong & Masumoto, 2000; Bai et al, 2006; Sewak, 2009; Gupta et al, 2014, 2017; Cheung et al, 2018; Han et al, 2021).

Remarks. This species has been reported by Balthasar (1963b) from the foothills of Pakistan. Arrow (1931) recorded this species from Myanmar, Sri Lanka and India. This species is distinct with an elongated middle tibia and a curved single or double tooth near the base beneath. This species shows wide distribution in the country. This species has good tolerance for shade. Good cow dung burial. Widely distributed in the forests. Attracted towards light. Prefers open grasslands and cultivated fields.

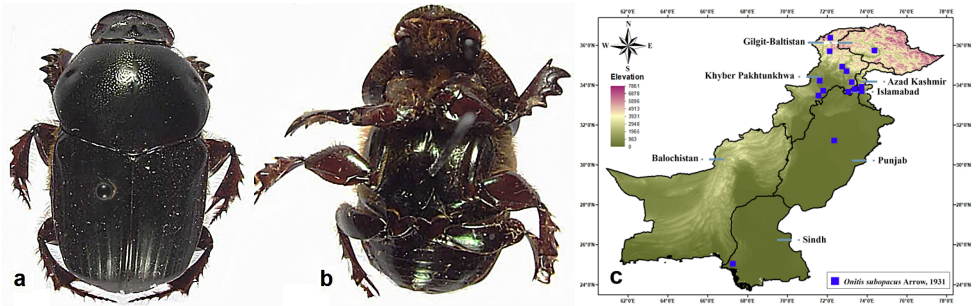

Figure 5. *Onitis subopacus* Arrow. a) dorsal, b) ventral habitus, c) regional distribution map in Pakistan.

Onitis virens Lansberge, 1875 (Figs. 6a-c)

Onilis virens Lansberge, 1875: 135. Type localities: India and Sri Lanka.

Onitis amplectens Lansberge, 1875: 136: Type locality: India.

Diagnosis. Body oval, moderately convex, and smooth in shape. Clypeus is elliptical, and closely transversely rugulose, with an intervallic carina separating it from the frons. Prothorax is fully punctured, with a median line in front, and a narrow median groove behind; there is a deep longitudinal pit on both sites of the middle of the basal margins, and the elytra have distinctly punctured intervals that are flat and striate. The pygidium is flat and punctured, and the metasternal shield is smooth. The front tibia is elongated, slandered curved with a long spine at extremity but short broad.

Material examined. PAKISTAN. Khyber Pakhtunkhwa: Abbottabad, 20.05.2009, 2♂♂, 3♀♀; Kohat, 26.07.2005, 4♂♂; Mansehra, 29.06.2006, 1♂, 5♀♀; Mardan, 03.07.2012, 3♂♂, 4♀♀; Nowshera, 12.05.2010, 2♂♂, 3♀♀; Peshawar, 13.08.2013, 4♂♂, 5♀♀; **I**slamabad: 02.07.2012, 2♂♂, 4♀♀, leg. M. Abbas. **CHINA.** Guangdong: Zhanjiang, 23.05.1984, coll. LIAO Tai-Bai, 1♂; Hainan: Luodai, 05.02.1973, coll. CUI Jing-Hai, 1♀; Guangxi: Wuming, 15.06.1956, coll. Unknown, 1♂; Slam. Cast., 1875-?-17, E. Candeze Coll., 1♀ (Type of *Onitis virens*) [ISNB]; Unknown, E. Candeze Coll., 2♀♀ (Type of *Onitis amplectens*) [ISNB].

Distribution. Pakistan. Khyber Pakhtunkhwa: Swat (Kanju); Sindh: Tharparkar (Siddiqui et al, 2014); Bangladesh, India, China, Myanmar, Belgium, Thailand, Vietnam and Laos (Hanboonsong & Masumoto, 2000; Bai et al, 2006; Sewak, 2009; Gupta et al, 2017).

Remarks. Arrow (1931) reported specimens of this species from the foothills of Pakistan. The identified specimens were distinguished on the basis of elongated front tibia with a blunt spine and the middle femur with a strong, rounded lobe at the middle trochanter on hind leg that was sharply toothed. In open pastures, this species is found in dung, and attract towards light. It lives in sandy, muddy soil; is rare on rocks; dislike human excrement; prefers 2-7 days old dung but occasionally may remain in the dung up to two weeks. Resemblance to *O. subopacus* and can be differentiated from the examining of the characters of protibia in *O. virens* 4th tooth of protibia blunt and joined with 3rd tooth while in *O. subopacus* protibia tridentate with a terminal blunt process.

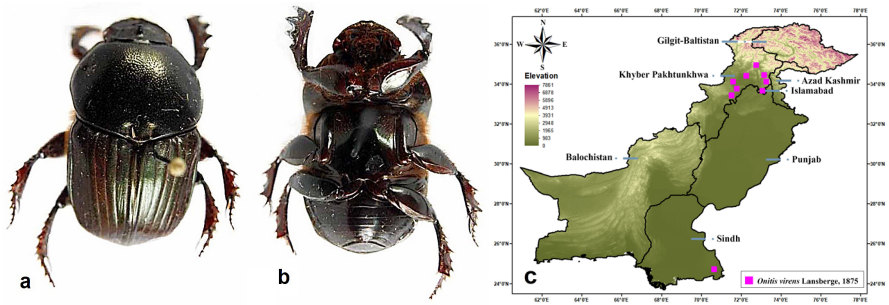

Figure 6. *Onitis virens* Lansberge. a) dorsal, b) ventral habitus, c) regional distribution map in Pakistan.

Genus *Cheironitis* Lansberge, 1875

Cheironitis Lansberge, 1875: 18. Type species: *Scarabaeus furcifer* Rossi, 1792.

Cheironitis arrowi Janssens, 1937 (Figs. 7a-c)

Cheironitis arrowi Janssens, 1937: 159. Type locality: India.

Diagnosis. Body oblong and somewhat depressed shape. Anterior margin of the clypeus strongly bilobed, reflexed, and separated from the frons by a short, transverse, clypeo-frontal carina with another carina behind it. Frons with some short erect setae. Pronotum is broader than elytra, unevenly rugose with very irregular punctures, coarsely and partly confluent in middle, finely and sparsely at lateral sides. Elytra are very lightly striate; the 2nd and 4th intervals are broad and rather flat; and the 3rd and 5th intervals are narrow, raised, and rather sharply carinate. Pygidium is lightly and sparsely punctate. Metasternum is longitudinally grooved, anteriorly clothed with erect hairs, punctuate very minutely and sparingly, and the lateral sides of metasternum and abdomen are clothed with fine setae (Figs. 7a-b).

Material examined. PAKISTAN. Khyber Pakhtunkhwa: Mardan, 27.06.2012, 3♂♂, 4♀♀; Malakand, 26.02.2005, 2♂♂; Mansehra, 29.08.2006, 3♀♀; Swat, 20.07.2010, 2♂♂, 3♀♀; Islamabad: 02.09.2012, 3♂, 3♀♀; Murree, 13.08.2013, 4♂♂, 5♀♀, leg. M. Abbas.

Distribution. Pakistan. Sindh: Karachi (Siddiqui et al, 2014); India, Afghanistan, Saudi Arabia, Nepal (Gupta et al, 2017).

Remarks. Sexually dimorphic species. Clypeus shiny and is finely and sparsely punctuated. The specimens were found in fresh cow and buffalo dung in the mountainous environments. Specimens were previously recorded from Pakistan, India, Afghanistan and Arabia (Balthasar, 1963).

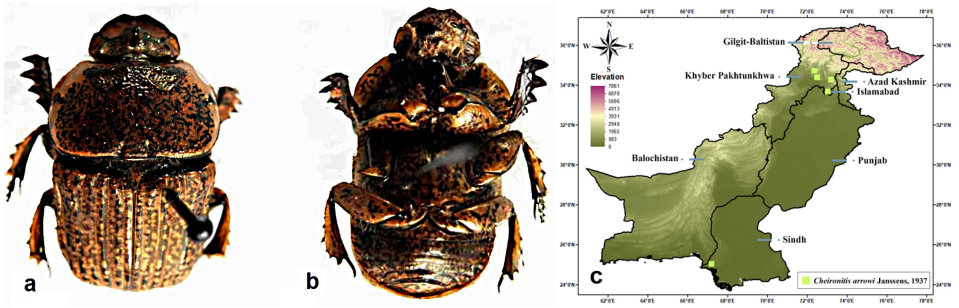

Figure 7. *Cheironitis arrowi* Janssens. a) dorsal, b) ventral habitus, c) regional distribution map in Pakistan.

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Research Article
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New Faunal Data for Black Flies (Diptera: Simuliidae), with the Evidence of COI Sequences, from Mediterranean Region of Türkiye

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ABSTRACT

The present study provides faunal data for Turkish Black flies (Simuliidae, Diptera) from the Central and Western Mediterranean Region in Türkiye. Among the specimens collected from 221 different running water sites, 17 species in 3 genera and 5 subgenera were identified. Two species, *Simulium* (*Nevermannia*) *ibleum* (Rivosecchi 1966) and *Simulium* (*Nevermannia*) *brevidens* (Rubtsov 1956), were recorded for the first time from Türkiye, and 5 additional species were reported from the study area for the first time. The morphological identifications were tested by phylogenetic analyzes using mitochondrial cytochrome oxidase I (COI) barcode sequences. The COI analysis results overlapped with the morphotaxonomic identification results for eight of the 15 species. The first genetic data of 4 species (for World) and 5 species (from Türkiye) were stored in GenBank (NCBI).

Keywords: Simulium, Prosimulium, Anatolia, DNA barcoding, Cox1.

Şirin, D. Ü., Fidan, E. C., Çalışkan, H. (2023). New faunal data for black flies (Diptera: Simuliidae), with the evidence of COI sequences, from Mediterranean Region of Türkiye, *Journal of the Entomological Research Society*, 25(2), 417-438.

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INTRODUCTION

Black flies (Diptera: Simuliidae) represent 26 genera and 2415 species (2398 living and 17 fossil) and have a wide geographic distribution occurring in all continents except Antarctica (Adler, 2022). These flies are key organisms in both aquatic and terrestrial ecosystems, but are perhaps best known for the blood-sucking habits of adult females. The females need blood to mature the eggs and this requirement makes this family important as biting pests and vectors of parasites and pathogens to humans and other warm-blooded animals (Crosskey, 1990). Due to the extensive cryptic speciation and high morphological homogeneity observed in Simuliidae, taxonomists emphasize that the diversity within the family has not been adequately revealed (Adler, Currie, & Wood, 2004; Andrade-Souza, Silva, & Hamada, 2017).

Türkiye, with its rich biodiversity, is still one of the countries in the Palearctic region with limited knowledge of its blackfly fauna. The first information about the Simuliidae in Türkiye was the description of *Simulium pulchripes* Austen, 1925 from Canakkale. However, until the 1990s, research on the family in Türkiye was almost nonexistent. Kazancı & Clergue-Gazeau (1990), in the first comprehensive study of black flies in the country, listed 21 species. 15 years later, 8 more species of the family were firstly reported from Anatolia by Şirin & Şahin (2005). Crosskey & Zwick (2007) published a checklist with 9 new records and distribution information, as well as all records known for the country up to that time. Çağlar & İpekdal (2009) listed 45 species and evaluated the Simuliidae fauna of Türkiye by comparing it with neighboring countries. Şirin, Çalışkan, & Şahin (2015) reported 17 species from the Turkish Thrace, with one species newly recorded for the country. In 2015, a new species belonging to the genus *Prosimulium* was described by Adler & Şirin (2015), and another new one of the genus *Metacnephia* was described by Şirin & Adler (2015). Başören & Kazancı (2016) published a checklist for the species of Simuliidae of Türkiye. An understanding of the Turkish simuliid fauna also benefited from cytotaxonomical studies (Adler & Şirin, 2014; Adler et al, 2015). In recent years, there has been an increase in studies on the fauna of Simuliidae in Türkiye. Furthermore, information about the presence and distribution of these flies in many areas of the country is still limited. The latest edition of the world Simuliidae checklist reports 57 species in Türkiye (Adler, 2022). Additionally, Fidan & Şirin (2022) added a new record for the fauna. This number corresponds to about 2.4% of all species in the family and about 9% of the Palearctic fauna. It is possible that more species of this family live in the country.

Cryptic species are common in the Simuliidae family and morphotaxonomic methods may be insufficient in the identification of some species (Adler et al, 2004). *Simulium*, the largest genus of the family, comprises many species complexes (Adler, 2022). However, cryptic taxa can display key differences that are important for ecological and epidemiological reasons for taxa whose bioindicator and vector species are widespread, such as Simuliidae. Therefore, accurate identification at the species level is very important for biomonitoring and vector control programs. In recent years DNA barcode approach has been widely used in studies focusing on phylogeny, population genetics and phylogeography in simuliids and revealing cryptic

diversity (Ruiz-Arrondo et al, 2018). In DNA barcode studies, the gene cytochrome c oxidase subunit I (COI) is mostly preferred as a marker for species identifications of black flies, as in many other animals (Andrade-Souza et al, 2017).

In this study, it was aimed to determine the black fly species living in the Central and Western Mediterranean region of Türkiye by both morphotaxonomic and molecular taxonomic methods.

MATERIALS AND METHODS

Specimen collection

The study material consisted of 10605 larvae, 8792 pupae and 172 reared adults (99 males and 73 females) from 221 different running waters in the Central and Western Mediterranean region and is deposited in the Eskişehir Osmangazi University Entomology Collection. Larvae and pupae were collected into 80% ethanol for morphotaxonomic examination and absolute ethanol for molecular analyses. Reared flies with their pupal exuviae were fixed in 80% ethanol.

Sampling localities information and dates are listed in Table 1 and the positions of the sites are shown on the map in Fig. 1. Numbers on the map and in the Table 1 provide correlation with the site records listed for each species in result section.

Figure 1. Map of collecting sites for black flies in Central and Western Mediterranean Region in Türkiye.

Table 1. Collecting sites for black flies in the Central and Western Mediterranean Region of Türkiye.

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
1	Burdur	37°46'53.12"	30°22'38.91"	930	25.03.2015
2	Burdur	37°38'59.36"	30°17'4.00"	1126	25.03.2015
3	Burdur	37°38'53.35"	30°17'46.47"	1170	25.03.2015

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
4	Burdur	37°35'27.47"	30°16'46.93"	1251	25.03.2015
5	Burdur	37°33'54.21"	30°16'48.08"	1380	25.03.2015
6	Burdur	37°30'44.74"	30°18'59.11"	1379	25.03.2015 / 26.06.2015
7	Burdur	37°45'55.42"	30°23'58.20"	1121	26.03.2015 / 24.05.2015
8	Isparta	37°44'36.16"	30°28'58.42"	1269	26.03.2015 / 30.03.2016
9	Isparta	37°47'43.28"	30°46'16.06"	1581	26.03.2015
10	Isparta	37°51'34.85"	30°48'6.89"	1060	26.03.2015 / 25.05.2015
11	Isparta	37°43'13.32"	30°56'5.65"	1170	26.03.2015 / 25.05.2015
12	Isparta	37°41'18.34"	30°57'1.94"	12313	26.03.2015
13	Isparta	37°39'19.59"	30°58'37.95"	1186	26.03.2015 / 25.05.2015
14	Isparta	37°36'41.64"	30°59'21.27"	1070	26.03.2015
15	Isparta	37°35'47.65"	30°59'12.52"	1050	26.03.2015 / 25.05.2015 / 05.05.2016
16	Isparta	37°32'36.43"	30°58'44.18"	924	26.03.2015 / 25.05.2015 / 05.05.2016
17	Isparta	37°33'2.89"	30°51'2.06"	583	26.03.2015
18	Burdur	37° 7'29.30"	29°41'17.04"	1021	27.03.2015
19	Burdur	36°57'52.55"	29°46'55.08"	1371	27.03.2015 / 27.04.2015
20	Burdur	36°49'35.90"	29°42'3.43"	1490	27.03.2015
21	Antalya	36°50'2.08"	29°47'0.64"	1207	27.03.2015
22	Antalya	36°26'0.79"	29°36'2.17"	1310	28.03.2015 / 25.06.2015
23	Antalya	36°25'8.22"	29°35'54.93"	1219	28.03.2015
24	Antalya	36°19'19.87"	29°28'11.42"	973	28.03.2015
25	Antalya	36°14'51.70"	29°28'5.33"	720	28.03.2015
26	Antalya	36°28'36.94"	30° 6'13.05"	289	28.03.2015 / 23.05.2015
27	Antalya	36°28'35.07"	30° 5'49.04"	550	28.03.2015
28	Antalya	36°27'54.94"	30°20'23.74"	533	28.03.2015 / 28.03.2016
29	Antalya	36°28'5.17"	30°20'21.45"	552	29.03.2015
30	Antalya	36°30'0.46"	30°19'43.94"	750	29.03.2015 / 28.03.2016
31	Antalya	36°34'50.80"	30°22'2.07"	1130	29.03.2015
32	Antalya	36°39'24.75"	30°24'27.45"	1119	29.03.2015
33	Antalya	36°45'44.12"	30°27'5.76"	946	29.03.2015 / 25.04.2015 / 23.05.2015
34	Antalya	36°55'1.64"	31° 1'33.15"	53	30.03.2015
35	Antalya	36°58'37.57"	31°12'3.75"	34	30.03.2015
36	Antalya	36°46'58.14"	31°43'16.30"	158	30.03.2015
37	Antalya	36°48'55.70"	31°53'14.23"	773	30.03.2015 / 22.05.2015 / 24.06.2015
38	Antalya	36°52'39.45"	31°45'57.53"	487	30.03.2015 / 21.05.2015
39	Antalya	36°56'7.51"	31°45'11.29"	642	30.03.2015
40	Antalya	37° 2'16.98"	31°43'59.62"	934	30.03.2015
41	Antalya	37° 4'48.90"	31°39'24.55"	456	30.03.2015 / 23.06.2015
42	Antalya	37° 7'41.15"	31°47'53.43"	1224	30.03.2015
43	Antalya	37°10'59.07"	31°46'38.91"	1195	30.03.2015
44	Antalya	37°14'9.33"	31°46'11.78"	1233	30.03.2015
45	Konya	37°22'20.05"	31°43'0.50"	1430	30.03.2015
46	Konya	37°32'59.61"	31°34'0.50"	1192	30.03.2015
47	Burdur	37°14'57.71"	29°32'44.53"	978	1.05.2014
48	Burdur	37° 1'45.04"	29°23'14.99"	1176	1.05.2014
49	Burdur	36°59'14.31"	29°24'26.59"	1105	1.05.2014
50	Burdur	36°59'18.42"	29°32'59.44"	1248	1.05.2014
51	Burdur	36°58'51.34"	29°33'20.32"	1347	1.05.2014
52	Burdur	36°52'21.29"	29°26'18.55"	1470	1.05.2014
53	Muğla	36°49'38.58"	29°33'45.71"	1124	1.05.2014

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
54	Muğla	36°49'7.76"	29°39'1.84"	1231	1.05.2014 / 27.04.2015
55	Antalya	36°49'16.24"	29°47'37.27"	1416	1.05.2014
56	Denizli	37°29'5.26"	29°24'37.21"	873	2.05.2014
57	Denizli	37°30'53.18"	29°30'54.94"	1092	2.05.2014
58	Burdur	37°25'7.18"	28°47'47.12"	1232	2.05.2014
59	Burdur	37°20'21.13"	29°56'53.55"	1073	2.05.2014
60	Burdur	37°34'25.12"	30°25'2.63"	1166	2.05.2014
61	Burdur	37°38'47.94"	30°25'19.35"	1264	2.05.2014
62	Burdur	37°38'21.82"	30°33'37.43"	1113	2.05.2014
63	Konya	38°17'20.41"	31°26'20.34"	1481	22.04.2015
64	Konya	38°14'58.69"	31°21'48.41"	1611	22.04.2015
65	Isparta	37°56'1.51"	31°18'12.95"	1128	22.04.2015
66	Isparta	37°42'18.94"	31°25'51.01"	1169	22.04.2015
67	Antalya	37° 6'19.14"	30°56'23.68"	40	23.04.2015
68	Antalya	37° 8'53.89"	30°54'45.56"	56	23.04.2015
69	Antalya	37° 8'49.51"	30°55'58.39"	56	23.04.2015
70	Antalya	37°10'32.74"	30°56'35.69"	150	23.04.2015
71	Antalya	37°12'59.32"	30°57'36.87"	271	23.04.2015 / 25.06.2015
72	Antalya	37°13'47.95"	30°57'52.72"	301	23.04.2015 / 25.06.2015
73	Antalya	36°30'14.94"	32° 9'18.09"	380	24.04.2015
74	Antalya	36°30'9.31"	32°10'45.70"	618	24.04.2015
75	Antalya	36°26'35.09"	32°11'32.87"	26	24.04.2015
76	Antalya	36°25'56.04"	32°16'19.68"	40	24.04.2015
77	Antalya	36°29'29.71"	32° 9'0.95"	140	24.04.2015 / 24.06.2015
78	Antalya	36°28'33.18"	32°16'23.34"	182	24.04.2015
79	Antalya	36°15'25.71"	32°19'57.01"	35	24.04.2015 / 02.05.2016
80	Antalya	36°15'43.20"	32°22'49.86"	88	24.04.2015
81	Antalya	36°15'1.46"	32°24'6.90"	87	24.04.2015
82	Antalya	36°47'45.15"	30°30'9.78"	644	25.04.2015 / 23.05.2015
83	Antalya	36°46'33.08"	30°25'38.44"	972	25.04.2015 / 23.05.2015
84	Antalya	36°42'26.38"	30°26'43.55"	1233	25.04.2015 / 23.05.2015
85	Antalya	36°40'30.05"	30°25'46.20"	1127	25.04.2015
86	Antalya	36°32'53.74"	30°20'47.84"	979	25.04.2015
87	Antalya	36°28'52.78"	30°19'59.78"	524	25.04.2015 / 23.05.2015 / 28.03.2016
88	Muğla	36°28'38.43"	29°24'31.48"	122	25.04.2015
89	Muğla	36°29'2.56"	29°18'49.24"	81	25.04.2015
90	Muğla	36°37'31.33"	29°20'49.02"	119	25.04.2015 / 23.05.2015
91	Muğla	36°42'42.72"	29° 2'56.65"	13	26.04.2015
92	Muğla	36°43'51.83"	29° 1'28.97"	11	26.04.2015
93	Muğla	36°54'40.28"	28°46'22.40"	48	26.04.2015
94	Muğla	36°56'15.37"	28°48'35.53"	171	26.04.2015
95	Muğla	36°56'29.93"	28°47'59.28"	178	26.04.2015
96	Muğla	36°59'20.20"	28°38'19.88"	6	26.04.2015
97	Muğla	37° 0'54.09"	28°20'37.88"	15	26.04.2015
98	Muğla	36°43'6.74"	29°11'7.84"	439	27.04.2015 / 24.05.2015 / 26.06.2015
99	Muğla	36°45'33.96"	29°14'5.19"	526	27.04.2015
100	Muğla	36°43'38.02"	29°21'25.63"	180	27.04.2015
101	Muğla	36°46'45.61"	29°28'34.06"	1084	27.04.2015
102	Muğla	36°54'40.17"	29°39'44.46"	1230	27.04.2015
103	Burdur	37° 0'58.50"	29°43'25.34"	1074	27.04.2015

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
104	Antalya	37° 1'55.45"	29°59'48.62"	1520	27.04.2015
105	Antalya	37° 0'13.60"	30°15'35.75"	922	27.04.2015
106	Antalya	37°14'58.30"	30°14'0.49"	955	28.04.2015
107	Burdur	37°35'45.60"	30°30'39.96"	1050	28.04.2015
108	Burdur	37°38'51.34"	30°36'27.35"	1035	28.04.2015
109	Burdur	37°38'6.93"	30°36'56.61"	1021	28.04.2015 / 26.06.2015
110	Isparta	37°39'48.60"	30°40'6.40"	785	28.04.2015
111	Isparta	37°42'45.79"	30°39'12.33"	880	28.04.2015
112	Burdur	37°31'37.84"	30°45'23.96"	350	28.04.2015
113	Burdur	37°26'37.10"	30°47'1.53"	280	28.04.2015
114	Isparta	37°48'24.09"	30°55'3.83"	1170	29.04.2015
115	Isparta	37°48'51.10"	30°55'57.49"	1172	29.04.2015
116	Isparta	37°47'10.10"	30°58'48.74"	1190	29.04.2015
117	Isparta	37°47'50.05"	31° 0'36.60"	1205	29.04.2015
118	Isparta	37°43'59.06"	31° 1'29.46"	1165	29.04.2015
119	Isparta	37°40'54.11"	31° 1'38.22"	1103	29.04.2015 / 27.06.2015 / 05.05.2016
120	Isparta	37°55'40.44"	30°55'47.08"	951	29.04.2015
121	Isparta	38° 0'43.03"	30°57'50.03"	954	29.04.2015
122	Isparta	38°12'36.74"	31° 6'40.64"	1020	29.04.2015
123	Konya	38°16'36.80"	31°25'22.45"	1484	21.05.2015
124	Isparta	38°12'20.72"	31°15'46.96"	1104	21.05.2015
125	Isparta	38° 3'12.73"	31°24'10.12"	1191	21.05.2015 / 23.06.2015
126	Konya	37°31'6.95"	31°48'34.15"	1126	21.05.2015
127	Antalya	36°58'55.29"	31°43'36.99"	763	21.05.2015
128	Antalya	36°43'47.22"	31°35'41.85"	12	22.05.2015
129	Antalya	36°47'22.72"	31°51'41.73"	485	22.05.2015
130	Antalya	36°49'21.53"	31°59'34.61"	890	22.05.2015 / 24.06.2015
131	Antalya	36°45'38.41"	32° 1'28.47"	250	22.05.2015 / 24.06.2015
132	Antalya	36°37'5.80"	31°52'49.34"	89	22.05.2015
133	Antalya	36° 7'23.68"	32°34'24.47"	24	22.05.2015 / 08.05.2016
134	Antalya	36°29'33.22"	30° 3'58.43"	394	23.05.2015
135	Antalya	36°25'38.81"	29°55'25.66"	405	23.05.2015
136	Antalya	36°20'42.48"	29°48'1.23"	220	23.05.2015
137	Antalya	36°16'40.57"	29°43'30.55"	213	23.05.2015
138	Muğla	36°49'59.03"	29°10'29.57"	918	24.05.2015 / 26.06.2015
139	Muğla	36°51'27.23"	29°10'42.99"	1305	24.05.2015 / 26.06.2015
140	Denizli	36°56'6.71"	29° 8'24.98"	996	24.05.2015 / 26.06.2015
141	Denizli	36°59'2.34"	29°12'3.71"	827	24.05.2015 / 26.06.2015
142	Denizli	37° 6'6.65"	29°24'0.61"	1319	24.05.2015 / 26.06.2015
143	Burdur	37° 7'27.31"	29°29'48.24"	1097	24.05.2015 / 26.06.2015
144	Burdur	37° 9'22.58"	29°36'47.06"	959	24.05.2015 / 26.06.2015
145	Burdur	37°30'49.79"	29°43'35.11"	1159	24.05.2015
146	Burdur	37°39'10.97"	30°10'36.58"	914	24.05.2015
147	Isparta	37°27'44.06"	30°54'33.13"	330	25.05.2015
148	Isparta	37°33'52.62"	30°58'45.03"	940	25.05.2015
149	Isparta	37°33'16.01"	31° 8'8.88"	1389	25.05.2015
150	Isparta	37°33'41.08"	31° 8'0.82"	1380	25.05.2015
151	Isparta	38°11'30.50"	31°14'42.31"	1094	23.06.2015
152	Konya	37°36'50.42"	31°35'30.42"	1144	23.06.2015
153	Konya	37°23'35.65"	31°41'19.53"	1408	23.06.2015

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
154	Antalya	36°39'25.77"	31°52'30.26"	148	24.06.2015 /03.05.2016
155	Antalya	36°31'55.43"	32°18'57.36"	364	24.06.2015
156	Antalya	36°26'41.22"	32°12'52.27"	74	24.06.2015
157	Antalya	36°57'12.07"	30°57'55.22"	22	25.06.2015
158	Antalya	37° 7'44.00"	30°55'3.41"	62	25.06.2015
159	Antalya	37°11'17.86"	30°57'33.30"	177	25.06.2015
160	Antalya	36°54'55.92"	30° 3'24.00"	1257	25.06.2015
161	Antalya	36°52'20.69"	30° 1'3.58"	1158	25.06.2015
162	Antalya	36°37'9.51"	29°46'45.95"	1068	25.06.2015 / 06.05.2016
163	Antalya	36°33'33.86"	29°37'48.16"	1680	25.06.2015
164	Antalya	36°33'29.95"	29°37'57.18"	1500	25.06.2015
165	Antalya	36°23'20.88"	29°31'6.98"	877	25.06.2015
166	Burdur	37°30'54.97"	30° 4'37.87"	990	26.06.2015
167	Burdur	37°29'40.15"	30° 9'3.70"	1103	26.06.2015
168	Burdur	37°26'11.71"	30°15'9.27"	1292	26.06.2015
169	Isparta	37°48'43.08"	31° 0'45.64"	1202	27.06.2015
170	Isparta	37°48'40.02"	31° 5'3.93"	1331	27.06.2015
171	Isparta	37°47'57.81"	31° 6'52.83"	1257	27.06.2015
172	Isparta	37°43'26.17"	31° 8'23.99"	1252	27.06.2015
173	Isparta	37°42'58.60"	31°14'41.71"	1373	27.06.2015
174	Isparta	37°42'2.57"	31° 1'58.27"	1124	27.06.2015
175	Muğla	37°17'46.31"	28°10'9.74"	399	27.03.2016
176	Muğla	37°17'42.40"	28° 8'59.87"	363	27.03.2016
177	Muğla	37°11'23.15"	28°34'37.27"	790	27.03.2016
178	Muğla	37° 9'22.26"	28°34'10.19"	78	27.03.2016
179	Muğla	37° 2'17.25"	28°30'23.28"	100	27.03.2016
180	Muğla	37° 0'15.55"	28°33'1.41"	88	27.03.2016
181	Muğla	36°55'35.41"	28°49'42.45"	136	27.03.2016
182	Muğla	36°44'46.03"	28°59'27.59"	180	27.03.2016
183	Muğla	36°42'23.62"	29° 2'54.05"	22	27.03.2016
184	Muğla	36°31'27.30"	29°24'4.81"	228	28.03.2016
185	Muğla	36°30'5.62"	29°19'43.57"	85	28.03.2016
186	Antalya	36°39'1.55"	30°25'58.25"	1120	28.03.2016
187	Antalya	37°0'49.49"	30°49'47.99"	61	29.03.2016
188	Antalya	37°11'27.64"	30°47'50.91"	49	29.03.2016
189	Antalya	37° 3'9.65"	31° 6'15.93"	148	29.03.2016
190	Antalya	36°58'39.93"	31° 7'33.71"	47	29.03.2016
191	Antalya	37° 2'41.55"	31° 6'12.33"	155	29.03.2016
192	Antalya	37° 0'59.03"	31° 7'22.42"	64	29.03.2016
193	Antalya	37° 0'47.42"	31°11'49.91"	20	29.03.2016
194	Antalya	37° 3'19.78"	31°14'14.05"	95	29.03.2016
195	Antalya	37° 7'54.33"	31°12'36.87"	112	29.03.2016
196	Burdur	37°20'33.46"	30°48'31.64"	193	29.03.2016
197	Burdur	37°36'58.12"	30° 4'11.34"	862	30.03.2016
198	Burdur	37°40'53.64"	30° 1'3.61"	928	30.03.2016
199	Burdur	37°42'48.95"	30° 0'56.30"	1050	30.03.2016
200	Burdur	37°39'19.66"	29°49'24.53"	1085	30.03.2016
201	Burdur	37°37'19.88"	30°21'0.11"	1197	30.03.2016
202	Antalya	36°51'28.60"	31°25'53.02"	25	3.05.2016
203	Antalya	36°51'41.56"	31°33'59.46"	59	3.05.2016

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
204	Antalya	36°37'52.47"	31°54'37.97"	142	3.05.2016
205	Antalya	36°59'25.76"	30°33'53.47"	314	3.05.2016
206	Antalya	37° 1'38.06"	30°16'31.99"	920	4.05.2016
207	Antalya	37°26'35.35"	30°47'53.25"	306	4.05.2016
208	Isparta	37°34'52.34"	30°49'27.02"	404	5.05.2016
209	Burdur	37°12'43.82"	29°43'28.76"	1202	6.05.2016
210	Antalya	37°0'11.36"	29°56'36.81"	1405	6.05.2016
211	Antalya	36°52'34.42"	30° 1'17.48"	1161	6.05.2016
212	Antalya	36°34'39.01"	29°43'32.58"	1156	6.05.2016
213	Antalya	36°25'42.23"	29°36'21.08"	1250	6.05.2016
214	Antalya	36°18'11.03"	29°27'17.89"	1018	6.05.2016
215	Antalya	36°53'26.28"	29°39'43.39"	1080	7.05.2016
216	Denizli	37°14'42.01"	29°31'20.96"	920	7.05.2016
217	Denizli	38° 9'32.12"	29°38'47.76"	812	7.05.2016
218	Isparta	37°58'55.42"	30°58'10.96"	953	5.05.2016
219	Antalya	37°38'42.17"	30°59'31.66"	1189	5.05.2016
220	Antalya	36°49'49.58"	29°33'42.72"	1120	7.05.2016
221	Antalya	36°56'33.73"	29°38'13.10"	1400	7.05.2016

Identifications

Material was studied under a stereomicroscope (Leica MZ 16), according to methods described by Bass (1998) and identified by using the keys by Rubtsov (1956), Knoz (1965), Crosskey (2002), Bass (1998), Crosskey & Malicky (2001), Yankovsky (2003), Crosskey & Zwick (2007) and Jedlicka, Kudela, & Stloukalova (2004). The nomenclature follows that of Adler (2022).

DNA Extraction, polymerase chain reaction and sequencing

The cytochrome c oxidase subunit I (COI) gene region of mitochondrial DNA (mtDNA) was analyzed. We used GenBank accessions and new sequences acquired in this study. Total DNA was extracted via Macherey-Nagel Animal Genomic DNA Extraction Kit. The universal primers LCOI (5′-GGTCAACAAATCATAAAGAT ATTGG-3) and HCOI (5′TAAACTTCAGGGTGACCAAAAAATCA-3′) were used for amplification (Simon et al, 1994).

Polymerase chain reaction (PCR) was carried out in 50 µl volume; 0.2 µl from each primer (100 pm), 1 µl Deoxynucleotide solution mix (10 mM), 4 µl 50 mM MgCl2 (25 mM), 5 ml 10X Standart Taq reaction Buffer [containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl], 0.25 Taq DNA polymerase (New England Biolabs), and 3 µl of 50–70 ng sample DNA. PCR cycling parameters were as follows: denaturation at 95℃ for 30 sec, 35 cycles of 95℃ for 20 sec., annealing at 41℃ for 30 sec, elongation at 72℃ for 1 min 40 sec. and final extension at 72℃ for 5 min. Results were visualized with agarose gel electrophoresis including ethidium bromide stain. Sanger sequence analysis and purifications were carried out by Macrogen Europe (Amsterdam, the Netherlands).

Molecular Data Analysis

The analyses were conducted with 109 COI sequences from 19 species of Simuliidae. We used NCBI GenBank accessions (52 sequences+4 sequences as

outgroups) and 53 new sequences acquired in this study. All sequences were checked manually with SEQUENCER v. 4.1 (Gene Codes Corporation). The alignment was made with Mafft version 7 (https://mafft.cbrc.jp/alignment/server/), following the auto strategy. The number of conservatives, variable and parsimony-informative sites were calculated using MEGA7 (Kumar, Stecher, & Tamura, 2016). Haplotypes were determined by DnaSP v.5 (Librado & Rozas, 2009) and haplotype frequencies were calculated. Sequences are deposited in the Genbank database. The best-fit evolutionary model for our data matrix was estimated by jModelTest v.0.1.1 (Posada, 2008).

Aligned sequences were analyzed with Maximum parsimony (MP), with 100 random additions, nearest neighbor interchange (NNI) algorithm and heuristic search approach by PAUP Version 4.0b10 (Swofford, 2002), and Maximum Likelihood (ML) with raxmlGUIversion 1.5 (Silvestro & Michalak, 2012) and ML-rapid 1000 bootstrap option. MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) program was used for Bayesian phylogenetic inference (BI), with four simulations of Markov chains, 10 M generation and sampling every 100th generations and with 1000 trees discarded as burn-in. As outgroups we used two species of Blephariceridae: *Elporia barnardi* (GenBank acc. num: AF427037.1 and AF427038.1) and *Liponeura cinerascens cinerascens* (GenBank acc. num: MW181342.1 and MW181343.1). While the relationship between haplotypes was carried out through median-joining, MJ network approach Network 4.5.1.6 (http://www.fluxux-engineering.com, access date: 19.09.2022.) program, mutational distances between haplotypes were analyzed with SplitsTree v.4.11.3 (Huson & Bryant, 2006) program.

DNA sequence-based analysis TCS (Clement et al, 2000) and Automatic Barcode Gap Discovery (ABGD) programs were applied to the dataset for the species delimitation test. The distance-based test SpeciesID (speID) was conducted to estimate large range supported cluster numbers in the range of 0.5–7% threshold values (Shiyang, Vaidya, & Ng, 2006).

NCBI accession codes and registration data for each type of COI sequences used in the study are listed in Table 2.

Table 2. Species and the NCBI GenBank accession number of the COI sequences included in this study.

Species	Accession No	Country	Author
Prosimulium rachiliense Djafarov, 1954	OK073998	Türkiye	This study
	OK073996		
	OK073997		
	OK073991		
	OK073993		
	OK076952		
	OK076955		
	OK076957		
	OK076954		
	OK076713		
	OK066328		

table continued

Species	Accession No	Country	Author
Prosimulium hirtipes (Fries, 1824)	MF197685.1	Sweden	Kudela et al, 2018
	MF197686.1		
	KP861150.1	United Kingdom	Unpublished
	KP861151.1		
	KP861149.1		
	KP861148.1		
	KP861147.1		
Metacnephia subalpina (Rubtsov, 1956)	OK076953	Türkiye	This study
	OK076956		
Metacnephia lyra (Lundstrom, 1911)	KT278290.1	Sweden	Unpublished
	JQ220531.1	Finland	Unpublished
Simulium petricolum (Rivosecchi, 1963)	OK066351	Türkiye	This study
	OK066352		
	OK066353		
	OK066354		
	OK076714		
	OK066575		
	OK066576		
	OK066577		
	OK067201		
	GQ465967.1	United Kingdom	Unpublished
	GQ465951.1		
	GQ465952.1		
	GQ465950.1		
Simulium ibleum (Rivosecchi, 1966)	OK073994	Türkiye	This study
Simulium angustitarse (Lundstrom, 1911)	KP861164.1	United Kingdom	Unpublished
	KP861165.1		
Simulium brevidens (Rubtsov, 1956)	OK046460	Türkiye	This study
	OS046728		
	OK046735		
	OK047087		
	MG894322.1	Spain	Ruiz-Arrondo et al, 2018
	MG894229.1		
	MG894186.1		
	MG894181.1		
Simulium costatum Friederichs, 1920	OK047370	Türkiye	This study
	OK047418		
	OK047419		
	OK073995		
	OK047420		
	GU072929.1	Sweden	Unpublished
	GU072928.1		
	GU072927.1		
	GU072926.1		
Simulium cryophilum (Rubtsov, 1959)	OK047421	Türkiye	This study
	OK073992		
	OK073999		
	MG894307.1	Spain	Ruiz-Arrondo et al, 2018
	MG894188.1		
	MG599017.1	United Kingdom	Unpublished
	MG599016.1		

table continued

Species	Accession No	Country	Author
Simulium vernum Macquart, 1826	GU072982.1	Sweden	Unpublished
	GU072980.1		
Simulium bezzii (Corti, 1914)	OK047354	Türkiye	This study
	OK047368		
	OK047369		
	MK545146.1	Iran	Khanzadeh et al, 2019
	MK545145.1		
	MK545144.1		
	MK545143.1		
Simulium auricoma Meigen, 1818	OK148435	Türkiye	This study
Simulium kiritshenkoi Rubtsov, 1940	OK047478	Türkiye	This study
	OK047480		
	OK047479		
	MK545139.1	Iran	Khanzadeh et al, 2019
	MK545138.1		
	MK545136.1		
	MK545135.1		
Simulium ornatum sp-comp.	OK047481	Türkiye	This study
	OK047483		
	OK047482		
Simulium ornatum Meigen 1818	KP861038.1	United Kingdom	Unpublished
	KP861037.1		
	KP861036.1		
Simulium variegatum Meigen, 1818	OK066329	Türkiye	This study
	MG894323.1	Spain	Ruiz-Arrondo et al, 2018
	MG894321.1		
	MG894319.1		
	MG894301.1		
Simulium balcanicum (Enderlein, 1924)	OK047477	Türkiye	This study
	MH587353.1	Serbia	Đuknić et al, 2019
	MH587353.1		
	MH587354.1		
	MH549570.1		
	MH549569.1		
Simulium pseudequinum Séguy, 1921	MH587349.1	Greece	Đuknić et al, 2019
	MH587348.1		
	MH587350.1	Bulgaria	Đuknić et al, 2019
	MH587347.1	Croatia	Đuknić et al, 2019
	OK047270	Türkiye	This study
	OK041122		
	OK047269		
	OK046148		
	OK046149		
Simulium paraequinum Puri, 1933	OK039228	Türkiye	This study
	MT309529.1	Iran	Unpublished
	MT309530.1		
	MT309535.1		
	MT309537.1		

RESULTS

Morphotaxonomic analyses

A total of 19569 individuals (10605 larvae, 8792 pupae and 172 reared adults) collected from 221 rivers in the study area were evaluated and 17 species were identified. Information about the species is given below.

Prosimulium rachiliense Djafarov, 1954

Material examined: A total of 843 pupae, 2170 larvae, 5 males and 3 females collected from 67 sites (6, 7, 9, 10, 12, 13, 14, 15, 16,19, 20, 21, 22, 23, 24, 25, 28, 29, 30, 31, 32, 33, 40, 42,43, 44, 45, 46, 50, 52, 54, 55, 57, 63, 64, 65, 73, 74, 77, 82, 83, 84, 85, 86, 87, 101, 102, 103, 104, 108, 111, 113, 115, 117, 119, 121, 123, 142, 144, 149, 150, 163, 186, 190, 213, 215, 221) were examined.

Metacnephia subalpina (Rubtsov, 1956)

Material examined: A total of 259 pupae, 1007 larvae and 2 males collected from 24 sites (11, 13, 16, 23, 36, 45, 52, 54, 55, 64, 66, 72, 73, 101, 102, 111, 119, 121, 123, 124, 130, 149, 153, 221) were examined.

Simulium (*Eusimulium*) *angustipes* Edwards, 1915

Material examined: A total of 9 pupae and 10 larvae collected from 3 sites (8, 10, 79) were examined.

Simulium (*Eusimulium*) *petricolum* (Rivosecchi, 1963)

Material examined: A total of 1340 pupae, 978 larvae, 21 males and 15 females collected from 74 sites (1, 2, 3, 4, 7, 13, 19, 25, 33, 34, 35, 37, 38, 56, 57, 59, 62, 65, 69, 76, 77, 80, 81, 82, 83, 87, 90, 91, 92, 93, 98, 99, 100, 103, 105, 106, 112, 113, 119, 126, 127, 130, 131, 132, 134, 135, 136, 138, 140, 142, 143, 145, 146, 148, 154, 159, 165, 167, 168, 174, 176, 178, 179, 180, 182, 183, 193, 203, 204, 206, 215, 217, 218, 219) were examined.

Simulium (*Nevermannia*) *ibleum* (Rivosecchi, 1966)

Material examined: A total of 16 pupae and 17 larvae collected from 4 (6, 7, 22, 23) sites were examined.

Remarks: *Simulium ibleum,* new record for Türkiye, was previously described by Rivosecchi (1971) as a subspecies of *Simulium angustitarse* (Lundström, 1911). *Simulium angustitarse,* also reported from a few regions in Anatolia, is mostly distributed in Central and Northern European countries. Whereas, *S. ibleum* is found in Mediterranean countries (Adler, 2022). The gill filament branching angles and lengths of the pupae in our material, the shape of the postgenal cleft, the structure of the hypostomal and mandibular teeth, and the morphology of the ventral plate extracted from mature male pupae are confirmed with the desription of *S. ibleum* in Rivosecchi (1971).

Simulium (*Nevermannia*) *brevidens* (Rubtsov, 1956)

Material examined: A total of 60 pupae, 93 larvae and 1 male collected from 13 sites (37, 40, 71, 77, 82, 83, 86, 95, 98, 117, 119, 129, 139) were examined.

Remarks: *Simulium brevidens* is a member of the *S. vernum* species group, the largest species group in the genus *Simulium* and new for Turkish fauna. The distinctive features of this species in the pupal stage are the pattern and length of the anterior projection of the cocon, the shape of the common stalk of the gill filaments and the branching directions, and the double branched structure of the pupal thoracic trichomes. All these characters are observed in our pupae. Similarly, the shape of the postgenal cleft of the larvae confirms to the description of the species given by Knoz (1965).

Simulium (*Nevermannia*) *costatum* Friederichs, 1920

Material examined: A total of 34 pupae and 35 larvae collected from 8 (11, 16, 27, 28, 33, 63, 164, 210) sites were examined.

Simulium (*Nevermannia*) *cryophilum* (Rubtsov, 1959)

Material examined: A total of 23 pupae, 20 larvae and 2 males collected from 5 sites (15, 26, 33, 81, 82) were examined.

Simulium (*Nevermannia*) *vernum* Macquart, 1826

Material examined: A total of 29 pupae, 3 larvae, 1 male and 1 female collected from 2 sites (11, 44) were examined.

Simulium (*Simulium*) *bezzii* (Corti, 1914)

Material examined: A total of 142 pupae, 115 larvae, 7 males and 7 females collected from 18 sites (9, 10, 52, 56, 57, 61, 83, 85, 141, 142, 194, 196, 199, 211, 212, 218, 219, 220) were examined.

Simulium (*Simulium*) *kiritshenkoi* Rubtsov, 1940

Material examined: A total of 2885 pupae, 2967 larvae, 30 males and 27 females collected from 108 sites (3, 6, 7, 8, 10, 11, 13, 18, 22, 23, 25, 26, 27, 34, 35, 36, 38, 50, 51, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 69, 71, 75, 77, 79, 82, 83, 87, 91, 92, 93, 95, 96, 98, 100, 103, 106, 107, 108, 109, 112, 117, 119, 123, 124, 125, 126, 129, 130, 131, 133, 134, 135, 136, 138, 139, 140, 141, 142, 143, 144, 145, 148, 150, 151, 152, 153, 154, 161, 162, 166, 168, 169, 170, 171, 172, 175, 176, 181, 183, 185, 188, 191, 197, 198, 199, 200, 201, 203, 204, 205, 209, 210, 212, 213, 214, 215, 220, 221) (5, 16, 19, 31, 70) were examined.

Simulium (*Simulium*) *ornatum* species complex

Material examined: A total of 70 pupae, 90 larvae and 1 female collected from 5 sites were examined.

Remarks: This species belongs to the *S. ornatum* species group, and was found at 5 different localities. It is very similar morphologically to *S. kiritshenkoi* but differs in the common stems and height of the pupal gill filaments. Since we did not have suitable material to examine the male genitalia, it could not be determined which of the species in the species group.

Simulium (*Simulium*) *variegatum* Meigen, 1818

Material examined: A total of 1323 pupae, 1385 larvae, 11 males and 7 females collected from 56 sites (7, 8, 11, 13, 16, 17, 19, 23, 26, 27, 28, 29, 30, 31, 32, 33, 36, 38, 39, 41, 71, 73, 74, 77, 81, 86, 87,

88, 89, 91, 95, 101, 110, 113, 118, 119, 120, 122, 124, 125, 130, 131, 139, 144, 145, 147, 148, 150, 153, 155, 170, 173, 177, 186, 215, 221) were examined.

Simulium (Obuchovia) auricoma Meigen, 1818

Material examined: A total of 15 pupae, 22 larvae, 2 males and 2 females collected from 10 sites (9, 15, 38, 73, 101, 119, 131, 169, 174, 201) were examined.

Simulium (Wilhelmia) balcanicum (Enderlein, 1924)

Material examined: A total of 231 pupae, 256 larvae and 1 male collected from 19 sites (47, 67, 75, 79, 93, 126, 128, 144, 145, 146, 158, 162, 166, 176, 183, 185, 193, 217, 220) were examined.

Simulium (*Wilhelmia*) *paraequinum* Puri, 1933

Material examined: A total of 533 pupae, 351 larvae, 9 males and 5 females collected from 19 sites (25, 35, 36, 47, 48, 49, 67, 68, 119, 133, 144, 157, 158, 183, 190, 197, 199, 209, 216) were examined.

Simulium (*Wilhelmia*) *pseudequinum* Séguy, 1921

Material examined: A total of 980 pupae, 1086 larvae, 7 males and 5 females collected from 75 (6, 7, 8, 13, 19, 56, 57, 59, 69, 75, 76, 77, 78, 79, 81, 83, 90, 91, 92, 93, 96, 97, 98, 100, 109, 112, 117, 126, 128, 131, 132, 133, 134, 136, 137, 141, 142, 145, 146, 154, 156, 160, 162, 165, 166, 167, 169, 172, 175, 176, 180, 182, 184, 185, 187, 188, 189, 192, 193, 194, 195, 200, 202, 203, 204, 205, 206, 207, 208, 212, 215, 217, 219, 220, 221) sites were examined.

Phylogenetic analyses

We created our dataset with 19 Simuliidae species (15 species from the western Mediterranean and 4 species from NCBI) and a total of 109 COI sequences from them were used for phylogenetic analyses. All 109 files were checked with the BLAST program for correcting the gene region after alignment. The sequences changed between 580 bp and 650 bp before they were organized into data blocks with MEGA7. As a result, data sets of 105 sequences (with 4 outgroup sequences) and 560 base pairs were obtained. Of these, 285 sites were conservative, 275 were variable, and 209 were parsimony-informative. The file was converted into different formats for use in phylogenetic analysis with MEGA, DnaSP, Mesquite and DAMBE programs. Of 87 haplotypes gathered from the data block, four belonged to outgroup sequences and 76 were unique (each individual created a haplotype), whereas 11 were shared within species (two or three specimens of same species created haplotypes). Haplotype diversity was calculated as 0.9951 by DnaSP v.5.The jModelTest v.0.1.1 suggested the evolutionary model as GTR+I+G (general time reversible+ invariable sites + gamma), according to AIC (Akaike Information Criterion), with p-inv = 0.4110 gamma shape = 0.5050.

All phylogenetic analyses (ML, MP, BI) resulted in similar tree topology, which can be viewed in the BI tree (Fig. 2). Eight of the species identified in the study (*Simulium balcanicum, S. paraequinum, S. pseudequinum, S. petricolum, S. ibleum, S. auricoma, S. bezzii* and *Metacnephia subalpina*) were branched as separate monophyletic species in the tree, agree with the morphotaxonomic identifications. Species

delimitation tests also confirmed the results for these species. On the other hand, for the other 7 species (*Simulium brevidens, S. cryophilum, S. ornatum, S. ornatum species complex, S. costatum, S. variegatum* and *P. rachiliense*), both phylogenetic analyzes and species delimitation tests yielded interesting results.

First of all, it is surprising that all haplotypes of *Simulium brevidens* and *S. cryophilum* (both our own and NCBI records) were grouped together and were not separated as two different species in all species delimitation tests. A similar result was observed for *S. kiritshenkoi* and *S. ornatum species* complex. The haplotypes of *S. kiritshenkoi* and *S. ornatum species* complex in our study material, and *S. kiritshenkoi* registered to NCBI from Iran and *S. ornatum* haplotypes registered to the UK were all grouped as a single species in the phylogenetic tree. All species delimitation tests also indicated that these haplotypes belong to a single species.

Another interesting result that had appeared in both phylogenetic analyzes and species tests was for *S. costatum*. The haplotypes of our *S. costatum* material branched separately from the haplotypes registered with the NCBI from Sweden. Furthermore, all species tests also confirmed that these are different species. Additionally, *P. rachiliense*, which is common in the study area, was separated as a monophyletic species in phylogenetic analyzes as expected from *P. hirtipes*, another species in the same species group obtained from NCBI. TCS and SpeID tests showed some *Prosimulium rachiliense* haplotypes as different species, but phylogenetic analyzes did not support this conclusion except for the “OK073991” coded haplotype. On the other hand, the “OK073991” haplotype, surprisingly emerged as a different species in both phylogenetic analyzes and species tests. There could be three possible explanations. The haplotype could be (i) ancestral haplotype, (ii) a cryptic species or two cytoforms suggested by Adler & Şirin (2014) for *P. rachiliense* Anatolian populations, and (iii) a pseudogene.

The last species that gave different results than expected in phylogenetic analyzes and species tests is *S. variegatum*. For it, a single haplotype of a single individual was included in the data set from the West mediterranean region. However, this haplotype was distinguished from the Spanish haplotypes which downloaded from NCBI with high branch support value in all three phylogenetic analyses. This result was also supported by the species delimitation tests.

According to the results of network analysis; the haplotypes shown in red in the middle, indicates the (Hypothetic) haplotypes that are not included in the study data set but should be found among the existing haplotype connections. As a result of the analysis, it is seen that the species do not share haplotypes with others and they are grouped similarly in the phylogenetic tree (Fig. 3). Similar results were obtained in the SplitsTree analysis, which was applied to support the Network analysis (Fig . 4).

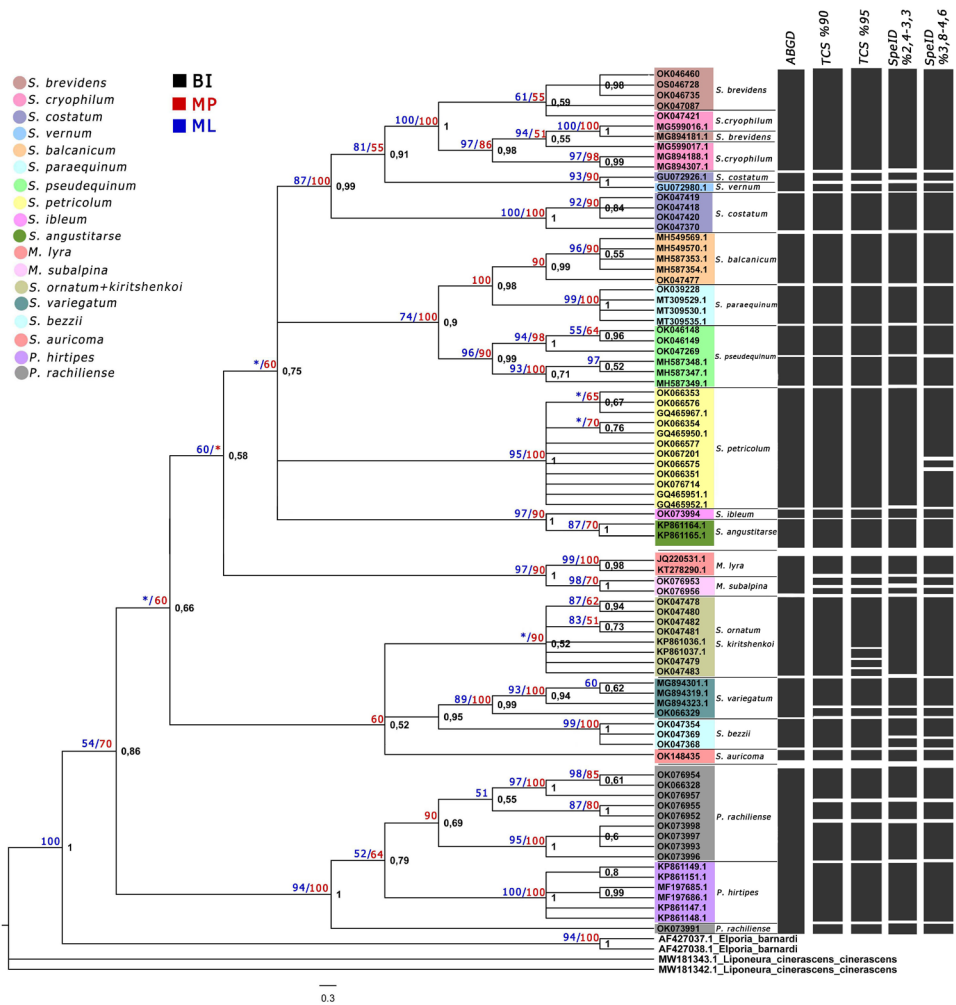

Figure 2. Phylogenetic tree for all analyses of Simuliidae from Central and Western Mediterranean Region of Türkiye. The numbers on the nodes indicate bootstrap values (MP-ML) or posterior probability values (BI). Black is for BI, red is for MP and blue is for ML respectively. (*) indicates 50% and below values or not supported by the respective analyses. Species delimitation tests are mapped on the tree (ABGD-TCS-SpeID).

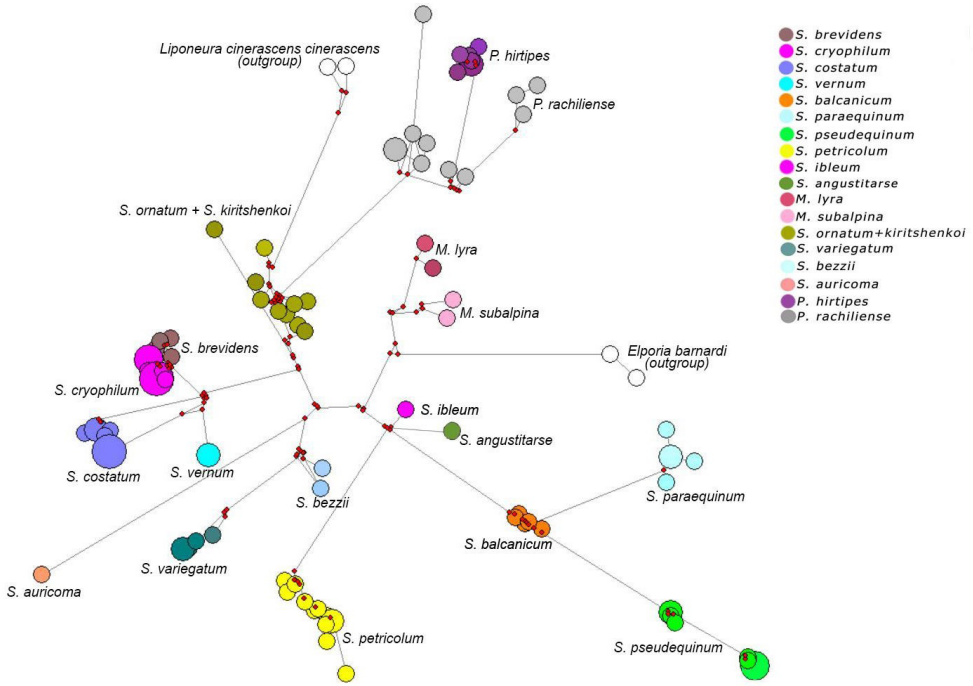

Figure 3. Haplotype network analysis created with COI 87 haplotype data set (Network programme).

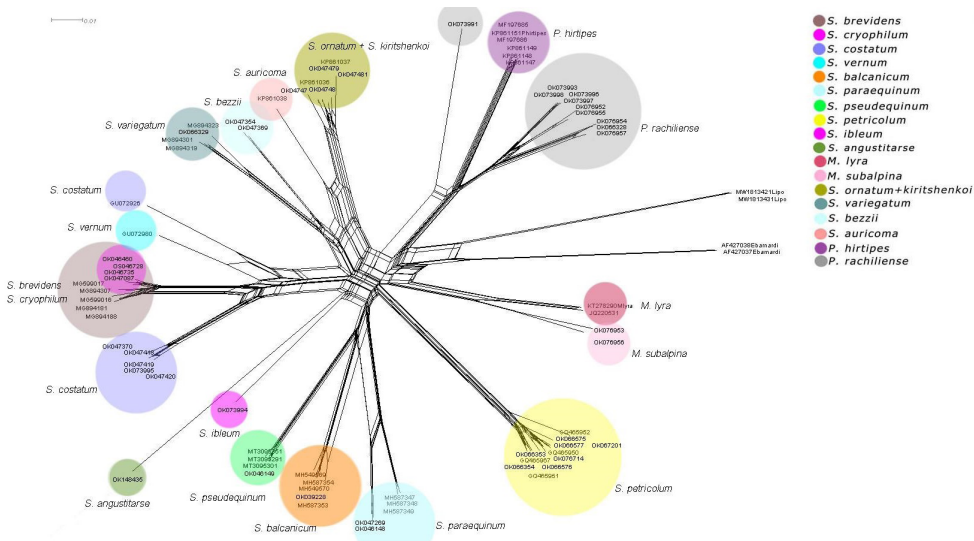

Figure 4. Haplotype network analysis created with COI 87 haplotype data set (Splitstree programme).

CONCLUSION

The results of the study have presented new contributions to the information about the Anatolian Simuliidae fauna. *Simulium ibleum* (Rivosecchi, 1966) and *S. brevidens* (Rubtsov, 1956), common species of other Mediterranean countries, were recorded in Anatolia for the first time in this study and the number of known species of the family in Türkiye has increased to 60. In addition, five species; *Metacnephia subalpina* (Rubtsov, 1956); *Simulium (Eusimulium) petricolum* (Rivosecchi, 1963); *Simulium (Nevermannia) costatum* Friederichs, 1920; *Simulium (Simulium) kiritshenkoi* Rubtsov, 1940 and *Simulium (Obuchovia) auricoma* Meigen, 1818 were reported for the first time in the Central and Western Mediterranean region and new locality records have been provided for other species as well. The species identified in the region are common in the central and southwestern Palearctic (Adler, 2022), as expected (Adler, 2022). *S. kiritshenkoi* was the dominant species found in 109 localities in the region. *S. pseudequinum* (83 localities) and *S. petricolum* (74 localities) also were extensively collected in the study area. The abundance of these three species may be due to the fact that they have multiple generations per year and wide habitat preferences.

Phylogenetic analyses performed with COI sequences confirm morphotaxonomic identifications of 11 species, while revealing some problematic situations for others. For example, the COI data revealed that *S. ibleum* is a distinctly separate species from *S. angustitarse*, of which it is a subspecies before. However, the analyses failed to separate *S. cryophilum* and *S. brevidens* reported for the first time from Türkiye and not registered in the NCBI. Both species are in the *S. vernum* species group, but *S. brevidens* is distinguished from *S. cryophilum* and other members of the group in the pupa by the following characters: upper and lower gill filaments at an acute angle; thoracic tubercles smooth and round; dorsal projection of cocoon short and irregular; and thoracic trichomes dichotomously branched (Jedlicka et al, 2004). We also observed these characters in our material, although the analysis of the COI data did not reveal a clear difference in the species status of *S. brevidens* and *S. cryophilum*. We suggest that additional genes, total mitochondrial genome or microsatellite studies could be used to investigate this taxonomic problem.

We also observed problematic results for *S. kiritshenkoi* and the species we identified as *S. ornatum* species complex. Many authors have stated that members of the *S. ornatum* species group are difficult to separate morphologically and genetically (Adler, Werner, & Kampen, 2021). Fidan (2020) investigated the phylogeny of Anatolian populations of the *S. ornatum* species group, using three different genes (COI, NADH2 and ITS1-2), and compared them with European populations. She concluded that *S. kiritshenkoi* and the *S. ornatum* have incomplete linage sorting and are not two separate species. It is seen that we have experienced a similar pattern in the study, when the phylogenetic tree and haplotype network analyzes are examined.

The COI data for the species identified as *S. costatum* in our study also differ from the data in the NCBI and indicate a different species according to phylogenetic and haplotype network analyses. In both network and phylogenetic analysis, *S. costatum*

haplotypes studied within this study were grouped separately. The morphological characters of the larvae used in the diagnosis of this species conformed to those given by Bass (1998). More extensive genetic analyses and morphotaxonomic studies are needed to determine if our material is a different species from *S. costatum*.

The most surprising result obtained in the study is the difference between the COI data from *S. variegatum* in our study area and the data in NCBI from Spain. The data point to two separate species, according to all species delimitation tests in our study. However, it is known that this species is easily distinguished from all other species of the family by two large thoracic bulges (patagia) anterodorsally in the pupal thorax, as in our material. This result suggests that the individuals from whom COI sequences were obtained may have been misidentified or the material using in DNA extraction may have been confused with another species. The result should be tested with more comprehensive material and genetic data.

The genetic information of 4 species *(Prosimulium rachiliense, Metacnephia subalpina, Simulium ibleum, S. auricoma)* was entered into GeneBank (NCBI) for the first time and the first NCBI record of 5 species (*S. petricolum, S. brevidens, S. costatum, S. cryophilum, S. variegatum*) from Türkiye were recorded through this publication.

Finally, our results emphasize that morphological and molecular data should be used together in order to accurately identify black fly species. This integrated approach will facilitate the identification of black fly species and the implementation of properly targeted vector control and pest management strategies. Our study area is a tourism region with considerable livestock activities. Thus, determining the presence of anthropophilic and mammophilic species such as *S. kiritshenkoi* and *S. pseudequinum* could contribute to the planning of biomonitoring programs to ensure human and animal health.

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AUTHOR CONTRIBUTIONS

ÜDŞ, ECF and HÇ collected the study material from running waters. ÜDŞ identified all species morphotaxonomically. ECF and ÜDŞ performed DNA extraction and phylogenetic analysis. ÜDŞ was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

The authors declare that they have no conflict of interest.

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Spatial Distribution and Abundance of *Chrysomya bezziana* in Jazan Province, Saudi Arabia Using GIS

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ABSTRACT

Myiasis caused by Calliphorid and Oestrid flies constitute a major threat to the development of livestock industry in Kingdom of Saudi Arabia. Increased veterinary concerns recently paid to the frequent reports on enteric pathogens caused by the larvae of these flies. Although the importance of sheep and goats in Saudi Arabia, the geographic distribution limits of Calliphorid and Oestrid infestation in caprine livestock has never been highlighted. ArcGIS software was used to assess the spatial distribution of myiasis causing flies in Jazan Province, Saudi Arabia. Implemented Evolutionary algorithms in maximum entropy (MaxEnt) was used to predict the distribution map for myiasis causing flies. Bioclimatic and topographic data layers from Worldclim was analyzed to estimate the percent contribution of variables predicting suitable habitats of flies causing myiasis. Field validation was occurred to evaluate the habitat suitability produced by the model. The predictive ecological niche model was found high with an AUC value of 0.95 and 0.93 for train and test occurrence records, respectively, with a standard deviation equal 0.032. About eighteen variables were found to contribute in spatial predictive occurrence of myiasis causing flies. Precipitation variables enhanced the model predictive power with (57.7%) in Jackknife test. Besides, elevation, NDVI and tree cover shared reduced effect in predicting myiasis causing flies distribution.

Key words: Myasis, MaxEnt, Spatial distribution modelling, Oestrid flies, Field validation

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INTRODUCTION

The Kingdom of Saudi Arabia is an extremely arid area, except for some coastal and mountains regions (Byrd & Castner, 2010). It lies in the southwestern part of Asia and extended through three ecological zones (Palearctic, Afrotropical and Oriental regions) (Clement, Hellier, Elberson, Staska, & Evans, 2007). Jazan Province, the southwestern part of the kingdom, is intermingled between three distinct topographical zones: Sarawat Mountains, Asir Plateau and Tihama lowlands (Mullen & Durfen, 2009). The diversity in topography alongside with significant variation in weather give the chance of wide variation in insect fauna in spatial and temporal scales especially for parasitic species such as myiasis causing flies (Nasser, Alahmed, & Shobrak, 2016; Al Ahmed et al., 2020).

Myiasis cause a serious reduction in livestock production in Southern Saudi Arabia including Jazan Province. Sporadic and consistent cases were recorded in previous studies from different areas in Saudi Arabia. The previous studies addressed seasonal fluctuation and abundance of different flies' taxa causing myiasis (Ansari & Oertley, 1982; Omar & Abdullah, 1992; Fatani & Hilali, 1994; Alahmed, 2002, 2004; El Azzazyz & El Metenawy, 2004; Abu Shehada, 2005; Alahmed, Al Dawood, & Kheir, 2006; Dawah & Abdullah, 2009; Zaglool et al., 2013; Setyaningrum & Al Dhafer, 2014; Hosni et al., 2019; Al Ahmed et al., 2020). At least four families have been incriminated in causing myiasis in the Kingdom, of which family Calliphoridae (blow flies) was the most important (Deeming, 2008). Thirty-four calliphorid species were reported from the Kingdom (Setyaningrum & Al Dhafer, 2014), but species of genus *Chrysomya* are known to cause myiasis in man and domestic animals (Deeming, 1996; Alahmed, et al., 2006, Nasser et al., 2021). In Saudi Arabia, *C. albiceps* and *C. megacephala* have been implicated as causing myiasis in camels (Gadallah & Bosly, 2006), whereas *C. albiceps* and *C. bezziana* were reported to cause myiasis in sheep and goats (Alahmed, 2002, 2004; Alahmed et al., 2006).

The significance of investigating the spatial distribution of blow flies stems from their importance in myiasis, pollination, and forensic tool (Kurahashi, 1989). Few previous studies tried to predict the geographic distribution of myiasis causing flies in response to climate variables. Although, many works concluded some correlations between infestation intensity and few climate parameters such as temperature and humidity (Fatani & Hilali, 1994) or between flies' development and temperature (Siddig et al., 2005), their findings were focal, lack consistent sampling design, and may not be applicable in large scale areas. To address limitations of previous studies, Geographic information system (GIS) and modeling tools were used to assess the geographic distribution range of flies causing myiasis in response to climatic, topographic, and land cover related variables in Jazan Province in other studies.

Geospatial mapping using GIS and modeling tools proved significant potentiality in predicting suitable habitats of insect pests in a large scale to a degree that is difficult or impossible using conventional ground survey (Sallam, Ahmed, Abdel-Dayem, & Abdullah, 2013; Al Ahmed, Naeem, Kheir, & Sallam, 2015; Hosni, Nasser, Al-Ashaal,

Magda, & Mohamed, 2020; Abou-Shaara et al., 2021). Recently, distribution modeling tools were widely applied using different approaches/methods such as generalized linear model (GLM), generalized additive model (GAM), multivariate adaptive regression splines model (MARS), hierarchical modeling, artificial neural network (ANN), random forest, genetic algorithm for rule-set prediction (GARP), maximum entropy and boosted regression tree (BRT) (Conley et al., 2014). The potentiality of each modeling tool in predicting species distribution depends on the sensitivity and specificity. Some of these tools depends on using presence/absence records such as BRT, however, the occurrence of absence and presence record at the same sampling site negatively affect the sensitivity of the analysis (Phillips, Anderson, & Schapire, 2006). In addition, most of the modeling tools is sample size dependent and their accuracy is positively correlated with the sample size. Nevertheless, MaxEnt is less sensitive to the sample size (Hosni et al., 2020).

The generation of risk maps for parasitic insects such as *Chrysomya bezziana* will help decision makers at the veterinary sector of Ministry of Agriculture to evaluate the risk of myiasis cases and give them the upper hand in its control. Consequently, this work aims at implementing modeling and GIS techniques in studying the spatial distribution of *Chrysomya bezziana* through Jazan province and giving notes on ecological and climatological parameters governing such distribution.

RESEARCH MATERIAL AND METHODOLOGY

Study area

Kingdom of Saudi Arabia (KSA) occupies approximately 2,250,000 km^2 of the Arabian Peninsula with a variable topography including areas of arid, semiarid, and forested landscape. (CDSI, 2010). The current study was conducted in Jazan Province, which is located in southwestern Saudi Arabia (Fig. 1) and includes ~13,432 km^2 (SGS 2012) inhabited by 1,365,110 people averaging 117 people/km^2 (CDSI, 2010). Topology and climate of Jazan can be categorized into three distinct sectors: the eastern Sarawat Mountains range 2,000-2,500 m Above sea level (a.s.l.) with an annual precipitation rate >300mm; hilly middle areas north to south with elevation range 400-600m a.s.l. and <300mm rain/year; and the coastal western plains with elevation <400m a.s.l. and little, if any, annual precipitation (Fig. 2). Overall, the Province has typically two rainy seasons, May-July and September-November (Ageel &Amin, 1997).

Jazan Province is divided into nine administrative districts (Fig. 1): Al Shaquiq (69,134 people/3632 km^2), Baysh 774, 421people /827 km^2) Sabya (22,8375 epeopl /1,983 km^2), Al Eidaby (60,799 people/1,290 km^2), Abu Areish (197,112people /927 km^2), Al Ardah (76,705people /852 km^2), Jazan (157,536 people/887 km^2), Ahad Al Msarhah (110,710 people/1,348 km^2), and Farasan (17,999 people /686 km^2) (CDSI 2010) (Fig. 3).

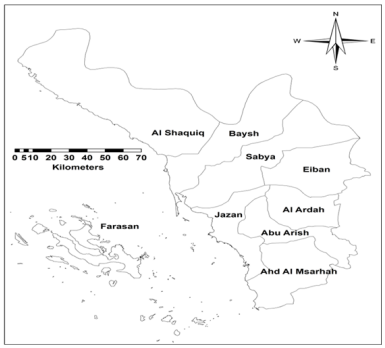

Figure 1. Map of Jazan Province and its administrative districts.

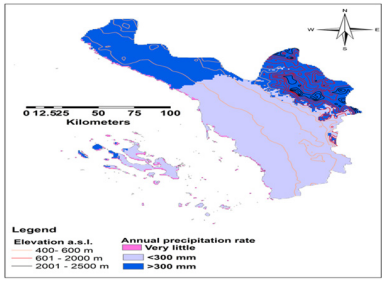

Figure 2. Map of Jazan Province representing different elevation levels and annual precipitation rate.

Spatial distribution of myiasis causing flies using Geographical Information System (GIS).

The adult flies were trapped using Red Top Fly Catcher (Matthew Hicks, Ashmoat Ltd, Suffolk, U.K.) baited with decomposed beef liver while the larvae were collected from infected animal cases (Camels, Goats and Sheep) with forceps. The adult flies were dried and preserved on separated plastic petri dishes, on the other hand the larvae were firstly preserved on 70% ethical alcohol and then some sample mounted on the slide for identification. The collected samples were identified according to Zumpt (1965), Hall & Smith (1993), Deeming (2008),Rognes (2002) and Setyaningrum & Al Dhafer (2014). To identify the spatial distribution of myiasis causing flies, occurrence records of predominant adult and larval flies causing myiasis and spatial statistics tools in ArcGIS software were used. Characterization of suitable habitats for myiasis causing flies and model its spatial distribution in response to climate, land cover and topographic variables, nineteen bioclimatic variables (11 layers of temperature and 8 precipitation indices) and elevation layer were obtained from the WorldClim database ver.1.4 (www.worldclim.org) (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). These layers are available at a 30 arc-seconds (~1km) resolution. Also, aspect ratio, slope, curvature, and hill shade were developed from the digital elevation model (DEM) using ArcGIS ver. 10.5.

Since vegetation has a significant importance for grazing sheep and shepherding activities, vegetation data was used in our model to assess the association between myiasis and type of vegetation. Therefore, normalized vegetation index (NDVI) was used as indicator for the vegetation type that may be associated with myiasis records. Also, it was reported in previous investigations that infestation with myiasis may be associated with certain land cover types; therefore, land cover data layer was included in our model. In addition, from the standing point that larval stage of myiasis causing flies need to pupate in soil, the distribution of emerged adult flies may be correlated with the soil type. Thereby, we used soil data layer to figure out the dependency of dipterous flies' distribution on soil as well.

Total of thirty-one bioclimatic, topographic, vegetation, soil types and surface water layers were then clipped to match dimensions of Jazan Province and saved as ASCII grids using Model Builder in ArcGIS software v.10.5 Jazan Province digital maps representing boundary, administrative districts were imported from Diva-GIS spatial database (http://www.diva-gis.org/Data).

Ecological niche model of myiasis causing flies

Maximum Entropy (MaxEnt) software v. 3.4 (Phillips et al*.,* 2006; Phillips & Dudik, 2008) was used to assess the spatial distribution and predict the ecological habitats of myiasis causing flies over the study area. The software uses only the occurrence records of recorded flies either adult or larvae and model their spatial distribution in response to the climatic and non-climatic variables.

The software was configured to the "Auto Features" mode as suggested by Phillips and Dudik (Elith et al., 2006), the logistic output format, and ASCII output file type. Since our model was counting on the occurrence records, the model was run with almost ~ 10,000 background data to represent pseudo-negative data beside the positive records we collected during the field visits.

MaxEnt reduces the duplicate records within ~1km of the same cell size (Zhou Munga, Minakawa, Githeko, & Yan, 2007). Records of myiasis causing flies (larvae and adults) were randomly partitioned for model evaluation into two subsamples: 75% of the records used for training and building up the model, and the remaining records (25%) were used for testing the model's accuracy. In this model, two indicators have been used to examine the performance accuracy. Extrinsic omission was evaluated at fixed threshold (10 percentile training presence) and the area under the curve (AUC) of the receiver operating characteristics (ROC). Although, MaxEnt uses only presence records data, negative (absence) data records of flies causing myiasis collected during surveillance were used to validate the produced prediction maps from MaxEnt. JackKnife analysis in MaxEnt was used to estimate the percent contribution of predicting variables to the model.

A total of 31 bioclimatic, topographic, vegetation, surface water, and soil types of data layers (Table 1) were used to predict the habitat suitability, and spatial distribution of myiasis causing flies. To represent the spatial range of myiasis causing flies, the

predicted habitat probability was categorized into three classes: very low-low (0-0.2), medium (>0.2-0.4), and high-very high (>0.4) using natural breaks in the symbology tools in ArcGIS software v.10.5.

Table 1. Percent contribution of the thirty-one variables used in the MaxEnt niche model to predict the spatial distribution and risk assessment of myiasis causing flies (adult and larvae) in Jazan Province, Kingdom of Saudi Arabia.

Variable	Variable name	Percent contribution
bio16	Precipitation of Wettest Quarter	39.3
bio7	Temperature Annual Range (BIO5-BIO6)	17.1
slope	Slope	15.2
alt	Elevation in meters	9.6
ndvi_sept	Normalized Difference Vegetation Index in September	3.9
bio13	Precipitation of Wettest Month	3.3
bio19	Precipitation of Coldest Quarter	3.1
bio14	Precipitation of Driest Month	1.6
tree	Tree cover	1.3
bio3	Isothermality (BIO2/BIO7) (* 100)	1.2
hillshade	Hill shade	0.8
bio1	Annual Mean Temperature	0.7
bio15	Precipitation Seasonality (Coefficient of Variation)	0.7
bio12	Annual Precipitation	0.5
curvature	Land Curvature	0.5
bio4	Temperature Seasonality (standard deviation *100)	0.4
ndvi_may	Normalized Difference Vegetation Index in May	0.4
bio11	Mean Temperature of Coldest Quarter	0.3
soil_jazan	Soil types	0
bio8	Mean Temperature of Wettest Quarter	0
bio6	Min Temperature of Coldest Month	0
lancover	Land cover	0
ndvi_march	Normalized Difference Vegetation Index in March	0
bio5	Max Temperature of Warmest Month	0
bio2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	0
bio18	Precipitation of Warmest Quarter	0
bio17	Precipitation of Driest Quarter	0
bio9	Mean Temperature of Driest Quarter	0
bio10	Mean Temperature of Warmest Quarter	0
aspect	Aspect ratio	0
water	Surface water streams/bodies	0

Field validation of the model:

The field validation was done at the best climatological condition of the Jazan region which known for its Afrotropical climate. So, A field survey was carried out for 15 days during January 2015, to evaluate the habitat suitability produced by our model. For the field validation, 77 sampling points were randomly selected and visited. Sampling design tool (SDT) in ArcGIS was used to randomly select field validation points representing the three-predicted habitat suitability. In this regard, the generated prediction maps from MaxEnt were used to select the validation points.

RESULTS

Ecological niche model of myiasis causing flies

The model was performed using 24 points (75%) for training and 7 points (25%) for testing. The predictive performance was found high with an AUC value of 0.95 and 0.93 for train and test occurrence records, respectively, with a standard deviation of 0.032. The fractional predicted area, at 10 percentile training presence was 0.136 and the test point's omission rate was 0.143. These points were classified as significantly better than random (P<0.0001). MaxEnt predicts 670.43 km^2 of very high suitable habitat (predicted risk probability >0.60), which is 4.99% of the total area of Jazan Province (Fig. 4).

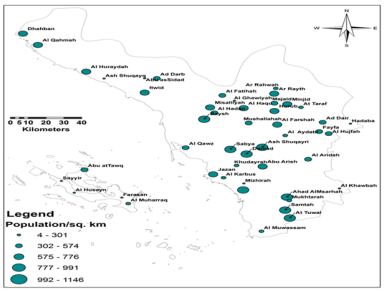

Figure 3. Map of Jazan Province representing distribution of human population.

Generally, the MaxEnt created model for myiasis causing flies supported two topographic sectors representing elevations ranges from 30-600 m. The very high predicted suitable habitat was found to be spotty in two main sectors with different elevations. The prediction map indicated consistent major distribution of high-very high suitable predicted habitats in hilly middle near the cities of Abu Areish, Al Eidaby, Sabya, Ahd Al Masarhah, and Al Ardah within the elevation range of ≈600 m a.s.l. Also, another area was indicated as high-very high suitable predicted habitat along the western coast of the Jazan Province at an elevation range 0-30m (Fig. 4).

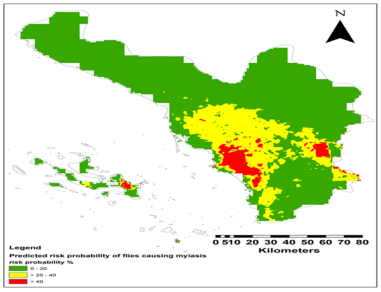

Figure 4. Predicted risk probability and spatial distribution range of *Chrysomya bezziana* through Jazan Province, Saudi Arabia.

Contribution of the variables to the model

Among the 31 variable layers used for spatial predictions using MaxEnt software, 18 variables were found to contribute in predicting occurrence of myiasis causing flies

(Table 1). The Jackknife test showed the precipitation variables significantly improved predictive power (57.7%) with the highest training gain compared to other environmental variables. The precipitation of the wettest quarter variable (bio16) presented the utmost training gain in the model. Furthermore, the temperature related variables shared a significant reduced training gain (19.7%) with precipitation in the model.

In addition, elevation, NDVI and tree cover shared reduced effect in predicting myiasis causing flies distribution (9.6, 4.3, and 1.3). Whereas the hill shade and curvature showed the least influence on spatial distribution of flies (0.8, and 0.5).

Field validation of the model

Out of 77 randomly selected field validation points, 53 sites (68.83%) were positive for dipterous myiasis causing flies (larvae and adult), whereas 24 (31.17%) were negative (Table 2). Of these positive collection sites, twelve (85.71%) in high-very high, nine (23.08%) in medium, and four (16.67%) in low-very low risk predicted areas were recorded.

Table 2. Risk probability percent in field validation of the model.

Risk probability %	Longitude	Latitude	Result
0 - 20	42.59	17.61667	negative
	42.5	17.54667	negative
	42.64	17.53667	negative
	42.76	17.50667	negative
	42.44	17.43667	negative
	42.87	17.35667	negative
	43.1	17.31667	negative
	42.94	17.16667	positive
	42.92	17.12667	positive
	42.98	16.95667	negative
	42.99	16.89667	negative
	42.96	16.84667	positive
	42.9	16.83667	positive
	42.9	16.77667	negative
	43.01	16.76667	negative
	42.87	16.73667	negative
	43.06	16.73667	negative
	43	16.66667	negative
	42.93	16.65667	negative
	43.06	16.62667	negative
	42.75	16.59667	negative
	42.97	16.56667	negative
	42.86	16.55667	negative
	43.07	16.55667	negative
> 20 - 40	42.57	17.43667	positive
	42.5	17.40667	positive
	42.63	17.40667	positive
	42.68	17.37667	positive
	42.75	17.35667	positive
	42.53	17.34667	positive
	42.62	17.34667	positive

Table 2. continued

Risk probability %	Longitude	Latitude	Result
	42.6	17.30667	positive
	42.48	17.29667	positive
	42.65	17.29667	negative
	42.57	17.28667	negative
	42.85	17.27667	negative
	42.5	17.26667	negative
	42.74	17.26667	negative
	42.43	17.24667	negative
	42.66	17.23667	negative
	42.55	17.22667	negative
	42.72	17.21667	negative
	42.52	17.19667	negative
	43.01	17.19667	negative
	42.76	17.17667	negative
	42.61	17.14667	negative
	43.03	17.14667	negative
	42.57	17.11667	negative
	42.7	17.11667	negative
	42.75	17.04667	negative
	43.01	17.01667	negative
	42.78	16.95667	negative
	43.07	16.94667	negative
	43.12	16.90667	negative
	43.16	16.81667	negative
	42.96	16.76667	negative
	43.16	16.76667	negative
	43.23	16.76667	negative
	42.8	16.73667	negative
	42.82	16.63667	negative
	42.8	16.54667	negative
	42.82	16.45667	negative
	42.84	16.38667	negative
> 40	42.77	17.12667	positive
	42.78	17.07667	positive
	42.6	17.04667	positive
	43.07	17.04667	positive
	42.68	17.02667	positive
	43.07	17.00667	positive
	43.11	17.00667	positive
	42.66	16.98667	positive
	42.73	16.98667	positive
	42.62	16.97667	positive
	42.65	16.92667	positive
	42.66	16.88667	negative
	42.71	16.84667	positive
	42.76	16.73667	negative

DISCUSSION

In the last two decades many investigations have demonstrated that GIS and modeling techniques tools are important tools in producing spatial prediction maps to

give better understanding of the ecological factors affecting transmission of infectious diseases (Dambach et al., 2012) and to study the spatial and temporal patterns of vector borne diseases (Kulkarni, Desrochers, & Kerr, 2010, Abdel-Dayem, Annajar, Hanafi, & Obenauer 2012, Sallam et al., 2013). The GIS and modeling tools were previously used potentially in mapping other vectors of diseases such as mosquito in Jazan Province (Sallam et al., 2013) and other regions (Zhou et al., 2007; Rohani et al., 2010). Similarly, these tools may potentially help in characterizing myiasis causing flies and their suitable habitats, and ultimately predicting the spatial risk of their occurrence. Accordingly, this will substantially help in producing risk maps to be used in decision making and implementing targeted control measures.

The habitat suitability model and risk maps summarize the distribution range of flies' suitable habitats. Moreover, a niche model for these myiasis causing flies in Jazan Province, KSA was built using most recent field data set on species occurrence and incidences of animal cases. In addition, the model was evaluated using independent field validation data records in assistance with the produced risk map. Moreover, the model examined the spatial heterogeneity of suitable habitats and resulting risk distribution range of myiasis causing flies.

This study elucidated the dependency of myiasis causing flies on climate and environmental variables (Precipitation, temperature, NDVI and tree) in Jazan Province. Previous studies either highlighted seasonal fluctuation and abundance of different flies' taxa (Abo Shehada 2005; Ansari & Oertley 1982; Alahmed, 2002, 2004; Alahmed et al., 2006; Dawah & Abdullah, 2009; El Azazy & El Metenaw, 2004; Fatani & Hilali, 1994; Omar & Abdullah, 1992; Setyaningrum & Al Dhafer, 2014; Zaghlool, Tayeb, Khodari, & Farooq, 2013) or the incidence rate and development of flies (Fatani & Hilali, 1994; Siddig et al., 2005). Although the importance of sheep and goats in Saudi Arabia, the geographic distribution range of Calliphorid infestation in livestock has never been highlighted. So, this work form the first study that applied the biogeographical tools in studying one of the most important myiasis causing fly of the Arabian Peninsula.

Sporadic previous studies addressed some correlations between few species of myiasis causing flies and some climate variables. Al Ahmed et al. (2006) highlighted the influence of seasonal activity of myiasis causing flies in livestock in Riyadh Region by the prevailing climatic conditions and availability of hosts. Similar studies concluded that variation in percentage of camels' infestations with *Cephalopina titillator* in the Eastern Region of Saudi Arabia were negatively correlated with monthly mean temperature and positively correlated with relative humidity (Fatani & Hilali, 1994). Moreover, they found that the percentage of infested camels and the mean monthly total number of larvae per camel showed two peaks during February (96.06% and 25.06 larvae per camel) and September (88.9% and 27.5 larvae per camel).

In addition, the pupal development of *C. bezziana* in Iraq was found to be limited by the low temperatures during winter, whereas the hot/dry summer conditions limit the geographic dispersal of adult flies. In these foci, the pupal development was fastest during the autumn months (Siddig et al., 2005). On the other hand, the continuous changes and variations in precipitation also lead to wider geographic

range of flies (Fischer, Thomas, Neteler, Tjaden, & Beierkuhnlein 2014), but may also reduce fly-related risks in other region. In regards to temperature, a small increase in temperature under laboratory conditions, can increase the hatchability of many of fly eggs (Shiravi, Mostafavi, Akbarzadeh, & Oshaghi, 2014; Roe & Higley, 2015). Also, higher temperatures increase the rate of trans-stadial development, so the insect proceeds to next stage in a shorter time (Shiravi et al., 2014). However, the positive influence of temperature on fly development may be limited as higher temperature will adversely affect their survival, such as when temperature reaches a survival threshold that can be lethal. Many fly species survive a wide range of temperature ranges spanning temperate, tropic, subtropics, and even Polar Regions. Also, temperature impacts the geographic range of flies in a local and regional scales. In the last decade, many flies species expanded their distribution range because of global warming and changes in the occurrence of suitable habitats in the trophic cycle (Cannon, 1998; Peňuelas, Filella, & Comas, 2002). The significance of the geographic expansion of flies to new areas, attributed to climate warming, causes nuisance and epidemiological situations through disease transmission or economic losses.

On the other hand, higher temperature may negatively influence the survival of flies and the development of disease pathogens inside their bodies. In regard to fly maggots that live on carcasses, the successive development of the maggots is solely dependent on the duration of the decay process, which eventually depends on climatic condition. In summer seasons, carcasses decay at much faster rate than in winter and spring. The increased temperature in summer speeds up the temporal succession of waves, whereas, in winter the rate of development of maggots slows down. Also, the maggot's activity leads to increased temperature of the corpse, which eventually results in quick decay. *Chrysomya megacephala* and *Chrysomya rufifacies* are very good examples of Calliphorids fly that are found in all the seasons of the year.

Accordingly, our model showed that humidity, temperature, and vegetation cover were found to be the play key role in the distribution of old-world screwworms (OWS) in Jazan province. Subsequently, application of Geographical Information System (GIS) tools is very valuable in understanding the distribution of OWS in relation to vegetation and watercourses. The very high and high predicted suitable habitats were shown to be sporadic in two main regions of this province representing two elevation ranges, the low and moderate elevations in Al Ardah, Sabya, Al Eidaby, Abu Areish, and Ahd Al Msarhah Districts. The animal incidence cases recorded during the validation phase of our model confirmed the risk map produced of this work. Further investigations need to be conducted in order to delineate the type of NDVI and tree cover highlighting types of plants and trees or shrubs correlated with the incidence of these cases.

It is well known that flies solely depend in part of their life cycle on the rainfall as a signature for the emergence of the adult stage from their pupae in soil. Thereby, precipitation shared the utmost gain in predicting establishment of larval and aerial stages of myiasis causing flies in infested areas. Precipitation during the wettest quarter (May-August), has the great influence on emergence of flies pupated in soil, in general, and especially myiasis causing flies. The influence of each climate and

environmental data layer on the model was evaluated via a Jackknifing procedure. Both precipitation and temperature are the two major predictors of flies. Since insects are ectothermic and depend on external temperatures to warm their body, air and land surface temperatures are believed to accelerate/suppress the development rate of insects (Lin & Lu, 1995, Murty, Rao, & Arunachalam, 2010). However, the contributions of NDVI and tree cover play a minor role in predicting flies' distribution alongside with precipitation and temperature.

The field validation collection points for our model demonstrated that the percentages of the positive sites corresponded with the predicted suitability values of the model. This can be attributed to the suitable niches that include wet soil and temperature ranges 25-35^{o}C. Negative records of myiasis causing flies were found in higher elevations, where farms are exposed to direct sunlight or high sanitation and hygiene are being practiced.

The ecological niche model was potentially proven to grasp the correlations between myiasis causing flies and their predicting variables. Moreover, it was very helpful in delineating the ecological variables that are necessary for supporting myiasis causing flies and their distribution range. However, other variables such as farms sanitation/hygiene, type of vegetation, and land use may contribute in determining the ecological niche habitats of these flies.

This novel distribution model and risk maps is the first in kingdom of Saudi Arabia. It potentially demonstrated that the distribution of myiasis causing flies is spatially clustered. In addition, different correlations between myiasis causing flies and climate and environmental variables have been highlighted. The novelty of the habitat suitability model represented that these myiasis causing flies preferred farms at low and moderate elevations (plateau areas) with relatively low and moderate precipitation at the wettest quarter and temperature range 25-35^{o}C. These suitable habitats occurred near the cities of Abu Areish, Al Eidaby, Sabya, Ahd Al Masarhah, and Al Ardah. The acquired data and risk map will be very effective tool on the hand of veterinary sector on Ministry of Agriculture on the Saudi Arabia in developing very effective control program for this fly and ensure the successfulness of the control strategy. Although MaxEnt ecological niche and land cover modeling are useful tools in predicting suitable habitats of dipterous flies in response to climate and environmental variables, more variables such as farms sanitation, type of vegetation, and land use need to be included in further study in Jazan Province.

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For faunistic research follow this order, Distribution:., Material examined:…, Host plant:….etc.

Example:
Sphex oxianus Gussakovskij, 1928
Distribution: Central and South West Asia, Afghanistan, Iran, Israel, Turkey (Bohart and Menke, 1976; Menke and Pulawski, 2000; Kazenas, 2001), Turkey: Artvin (De Beaumont, 1967).
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 ♀♀
Host plant: *Echinophora* sp.
Please use ♀, ♂ symbols. Please write upper genus categories with capital letters.

Illustrations: Illustrations, graphs, their caption or legends should form a separate, and a self-explanatory unit. Abbreviations in the legends should be explained but if there are too many, they should be included into a separate list. The original drawing and photographs should not be more than twice as large as when printed.

Morphological illustrations should include a scale bar. Photographs and electron micrographs should be in high-resolution JPEG file format (300 dpi). Drawings (black and white type) should be in TIFF format and their size should be no more than 10 MB. Graphs should also be in black and white and submitted in excel file format. Tables should include headings and explanations, and should be numbered consecutively. Their approximate position in the text should be indicated in the margin. Legends and titles of the graphs and tables should be in Arial with 12 type size. Please do not embed the figures, graphs and table into the text, and send them as supplementary files. In the text attribution to the figures should be given in parenthesis and must be abbreviate like this; (Fig.1).Figs. 1-10. *A. marriotti* sp. n. 1. Male (holotype), dorsal. 2. Female (paratype)

References:

Titles of manuscripts published in languages other than the major ones (English, German, French, Spanish, Portuguese, Turkish) should be an English translation (in parentheses) with an explanatory note at end, e.g. (in Russian). The list of references should be given at the end of the article and listed alphabetically, according to the following examples. All periodical names should be unabbreviated and italicized. In references, journal titles must be written in full (not abbreviated).

Journal Article

Beirne, B.P. & Young, D.A. (1953). The North American species of Cicadula (Homoptera, Cicadellidae). *Canadian Entomologist*, 85(1), 215-226.

Mitchell, J.A. (2017). Citation: Why is it so important. *Mendeley Journal*, 67(2), 81-95. Retrieved from https://www.mendeley.com/reference-management/reference-manager

Book

Steinmann, H.A. & Zombori, L. (1985). *An atlas of insect morphology*. (2nd ed.). Akadèmiai Kiadò, Budapest, Hungary.

Edited Book

Williams, S.T. (Ed.). (2015). *Referencing: A guide to citation rules* (3rd ed.). New York, NY: My Publisher

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Troy, B.N. (2015). APA citation rules. In S.T, Williams (Ed.). *A guide to citation rules* (2nd ed., pp. 50-95). New York, NY: Publishers.

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Mitchell, J.A. (2017, May 21). *How and when to reference*. Retrieved from https://www.howandwhentoreference.com.

Thesis

Ersoy, D. E. (2014). *Sündiken Dağlarının (Eskişehir) Aphodiinae ve Rutelinae Alt Familyalarının Sistematiği ve Faunası (Coleoptera: Scarabaeidae)* (Publication No. 12345678) [Doctoral dissertation - Master's thesis, Gazi University]. Council of Higher Education Thesis center. https://tez.yok.gov.tr/UlusalTezMerkezi/

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