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ENTOMON

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Note from the Chief Editor

Dear Entomologists,

Warm greetings to one and all!

The term of the present Executive Committee came to an end in March, 2017. There was election of new office bearers by the general body of the Association for Advancement of Entomology (AAE) functioning at the Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram, Kerala 695522 as per the bylaws. The general body was convened during April 2017 and elected new office bearers for the next three years (2017- 2019). The general body of AAE elected me as the chief editor for the second term.

The members were happy in its content, timely publication, NAAS score (4.48 for 2017), Zoobank registration, UGC recognition and CABI-UK repository of the ENTOMON. Also the new format of ENTOMON is gaining wide acceptance among the members, readers, peer reviewers and our stakeholders. This could be achieved only with the continuous support and cooperation of research entomologists from India and abroad. Prof. K.

Madhavan Nair, former Director, Centre for Information Technology, KAU has been instrumental in the development and maintenance of the website of ENTOMON/AAE. We are immensely thankful to him for the unstinted voluntary support in the progress of ENTOMON/AAE.

The Editorial Board of the ENTOMON has been reconstituted. We will strive hard for excellence of the ENTOMON and making an impact in the International arena with your valuable support and cooperation.

With the new team of Editorial Board presenting the ENTOMON 2017 - June (Volume 42: 2) issue.

Thanking the researchers, reviewers and institutions for their support.

INSECTS OBVIOUSLY AFFECT ALL PHASES OF HUMAN LIFE AND ENTOMOLOGISTS NEED TO WORK ON IT FOR THE BENEFIT OF HUMAN KIND AND ENVIRONMENT

Dr M.S. Palaniswami

Chief Editor

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Evaluation of *Cry IIa* transgenic chickpea lines for resistance to *Helicoverpa armigera* (Hubner) under controlled conditions

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ABSTRACT: Experiments were conducted to evaluate the effect of *Cry IIa* transgenic chickpea lines for resistance to *Helicoverpa armigera* using a cage technique. Results indicated that transgenic chickpea lines suffered significantly lower leaf damage as compared to non-transgenic lines. The larval survival and weight gained by the larvae was significantly reduced when *H. armigera* were fed on transgenic lines as compared to those fed on non-transgenic lines under glass house conditions. Across the seasons (2011-12 and 2012-13), the transgenic chickpea lines BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 exhibited high levels of resistance to *H. armigera* under laboratory conditions. Significant differences in grain yield were observed between transgenic and non-transgenic plants infested with *H. armigera* larvae. Since leaf damage was lower on transgenic chickpea plants, the dry matter weight, pod weight, seed weight and number of seeds formed were significantly more than on non-transgenic chickpea plants. In both the seasons, non-transgenic chickpeas yielded significantly lower compared to transgenic chickpeas.

KEYWORDS: Transgenic chickpea, *Helicoverpa armigera*, *Cry IIa*, Cage technique

INTRODUCTION

India imports about 1,85,000 metric tons of chickpea valued at US$ 94 m (FAOSTAT, 2011) The demand for chickpea is projected to double from 7 to 14 m tonnes by 2020. In the next 10 years the net import of chickpea will be close to 1.5 m tonnes to meet the domestic requirements. It is even more important for India, as the country's production accounts for 67 per cent of the global chickpea production, and chickpea constitutes about 40 per cent of India's total pulse production. It is a source of high quality protein for the poor people in many developing countries, including India. Chickpea yields are quite low, and have remained almost stagnant for the past 2 to 3 decades. It is valued for its nutritive seeds with high protein content (25.3–28.9 per cent).

Chickpea yields are low (400–600 kg ha^{-1}), because of several biotic and abiotic constraints, of which the pod borer, *Helicoverpa armigera* (Hubner)

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(Noctuidae: Lepidoptera) is the most important constraint in chickpea production (Manjunath *et al*., 1989). *Helicoverpa* females lay eggs on leaves, flowers and young pods. The larvae feed on the young leaves of chickpea and the young seedlings may be destroyed completely, particularly under tropical climates in southern India. Larger larvae bore into the pods and consume the developing seeds inside the pod. The losses due to *H. armigera* magnify under drought conditions. In addition to chickpea, *H. armigera* also damages several other crops such as cereals, pulses, cotton, vegetables, fruit crops and forest trees. It causes an estimated loss of US $ 2 billion annually, despite the use of US $ 500 million worth of insecticides to control this pest worldwide (Sharma, 2005).

In order to protect the crop from *H. armigera* damage, various pest management practices have been adopted by the Indian farmers. Efforts are being made to develop *H. armigera*resistant varieties by conventional breeding methods as well as modern biotechnological tools to develop transgenic chickpea varieties with resistance to this pest. The conventional control measures are largely based on insecticides. With the development of resistance to insecticides in *H. armigera* populations (Kranti *et al*., 2002), there has been a renewed interest in developing alternative methods of pest control, of which host plant resistance to *H. armigera* is an important component. The impact of genetically engineered insect-resistant crops on non-target organisms including biological control agents is one of the most widely discussed ecological effects.

Several studies have reported the direct and indirect effects of transgene products and the transgenic plants on the beneficial insects (Dutton *et al.,* 2003; Lovei and Arpia 2005; Sharma *et al.,* 2007, 2008 and Dhillon *et al.,* 2008). The *Bt* toxins are not transported to the phloem in some crops, and therefore, insect pests such as corn leaf aphid, *Rhopalosiphum maidis* (Fitch.) and the natural enemies feeding on it are not directly affected by the *Bt* toxins (Head *et al.,* 2001 and Dutton *et al.,* 2002).The present studies were undertaken to evaluate the effectiveness of transgenic chickpea lines resistant against *H. armigera.*

MATERIALS AND METHODS

Six transgenic and two non transgenic chickpea lines were evaluated for resistance to *H. armigera*. The plants were grown under greenhouse conditions (27 ± 5^0 C and 65 - 90% RH). Larvae of *H. armigera* used in the bioassays were obtained from a laboratory culture maintained at ICRISAT. The larvae were reared on chickpea based artificial diet (Armes *et al*., 1992) under laboratory conditions at 27^0C.

Cage screening: Each genotype was infested with neonate *H. armigera* at 30 DAE. Twenty neonates were released on the terminal branches of three plants in each pot using a camel hair brush. The plants were covered with a wire framed cylindrical cage (25 cm in diameter and 25 cm in height). The lower margin of the cage was pushed to a depth of 3 cm in the soil and covered with nylon bag of similar dimensions to prevent any escape of the larvae. There were three replications for each genotype. The experiment was monitored daily, and terminated when >80% of the leaf area was consumed in the control plants. The larvae were removed from the plants, placed individually in small plastic cups, and weighed after 4 h. The plants were then rated visually for the extent of leaf damage on a 1 to 9 damage rating scale (1 = <10% leaf area damaged; 2, 11-20%; 3, 21-30%; 4, 31-40%; 5, 41-50%; 6, 51-60%; 7, 61-70%; 8, 71-80%; and 9, >80% leaf area damaged). Data were recorded on leaf area damaged (visual damage rating), larval survival and larval weights.

Statistical analysis: The experiments were conducted in a completely randomized design (CRD) with three replications for each genotype. Data were subjected to analysis of variance by using GENSTAT version 14.1. The treatment means were compared by DMRT to know the significance of differences among the transgenic and non transgenic chickpea lines.

Figure 1. Evaluation of transgenic chickpeas for resistance to *H. armigera* under greenhouse conditions using cage technique (2011-2013)

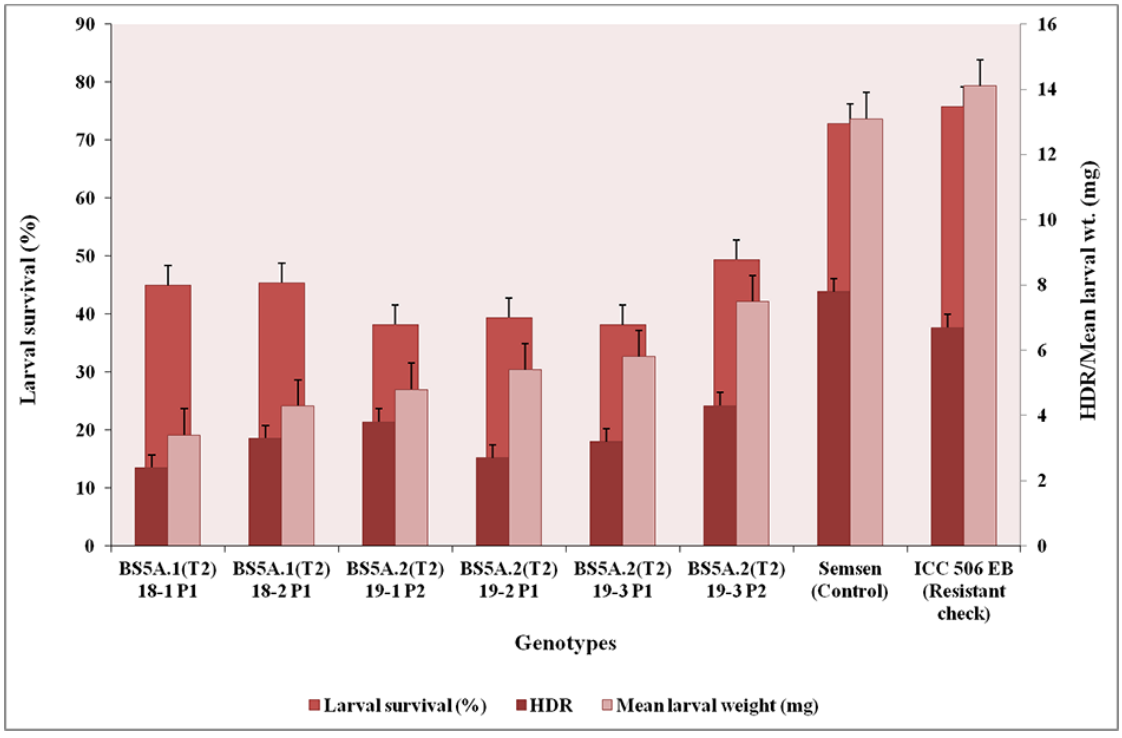


Figure 2. Agrnomic performance of transgenic chickpea lines (g/3 plants) with resistance to *Helicoverpa armigera* under greenhouse condition using cage technique (2011-2013)

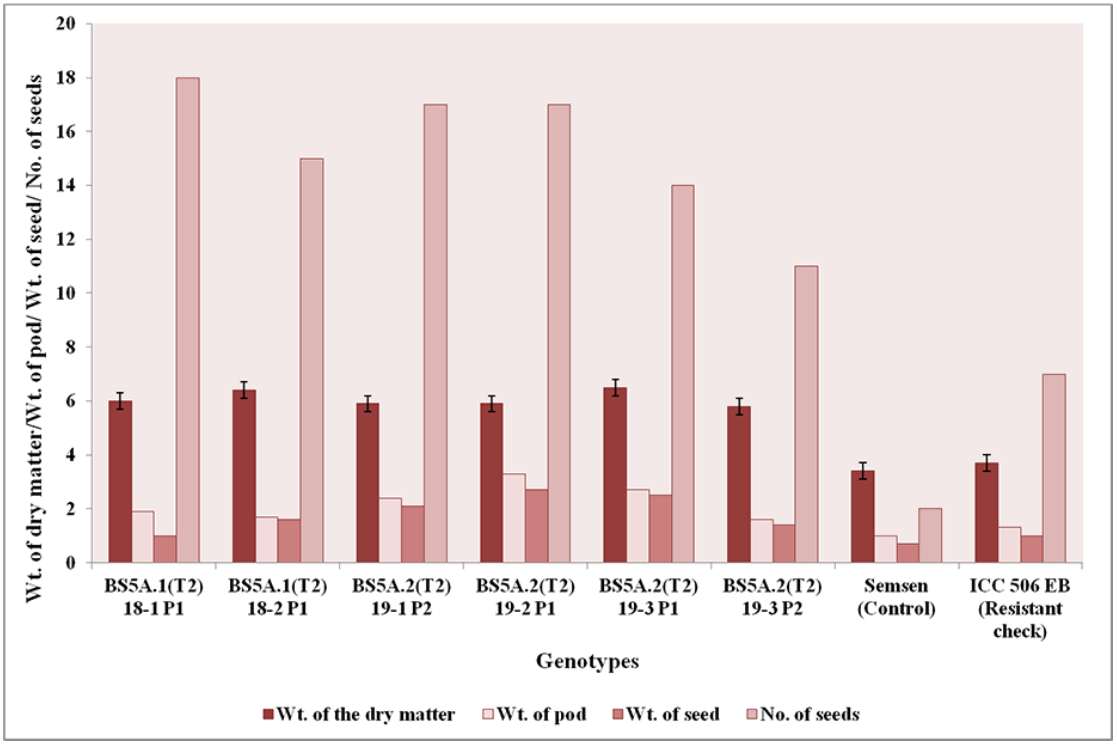
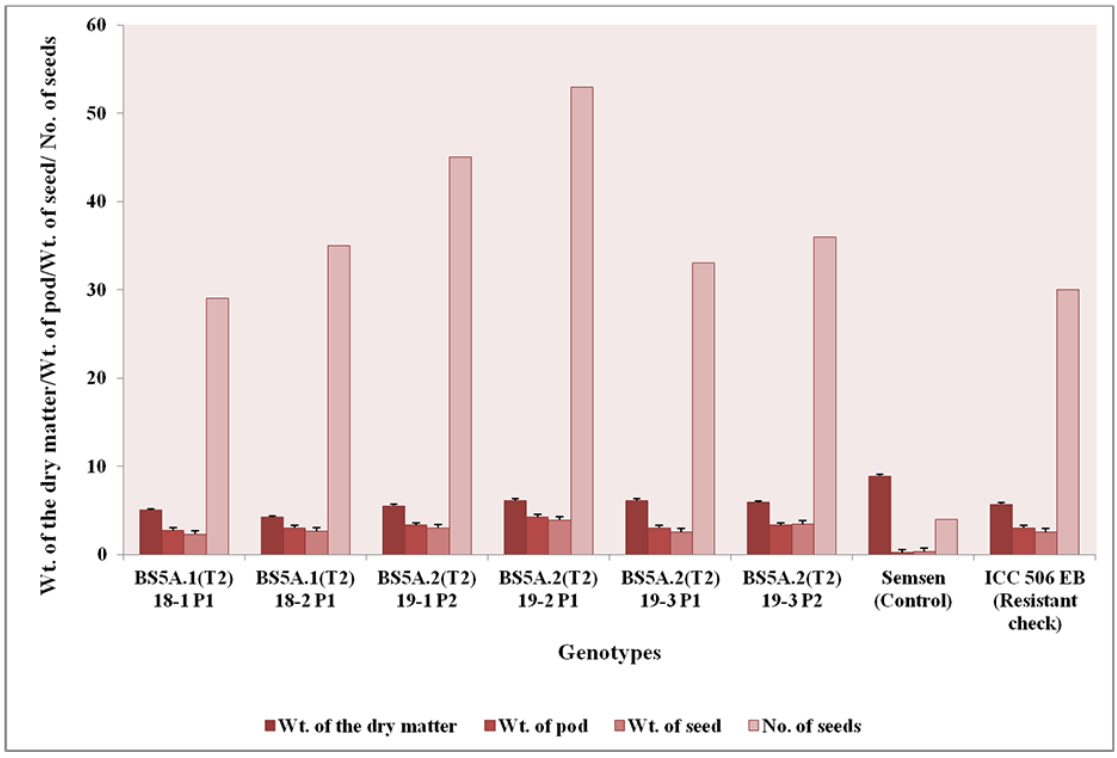


Figure 3. Agronomic performance of transgenic chickpea lines in un-infested plants (g/3 plants) under green house conditions (2011-2013)



RESULTS

Response of transgenic chickpea lines to damage under glasshouse conditions

During 2011-2012, leaf damage was significantly greater on ICC 506 EB (DR: 8.0) and Semsen (DR: 7.8) as compared to that on BS5A.2(T2) 19-2P1 (DR: 1.6). Among the transgenic lines tested, BS5A.1(T2) 18-2P1, BS5A.2(T2)19-1P2 and BS5A.2(T2) 19-3P2 suffered greater leaf damage (DR: 4.1, 4.4 and 4.3, respectively) than other lines tested. Larval survival was significantly greater on Semsen (75.7%) and ICC 506EB (72.3%) as compared to that on the transgenic plants of BS5A.2(T2) 19-3P1 (35.0%). Among the transgenic chickpea lines tested, significantly greater larval survival was recorded on BS5A.1(T2) 18-1P1 (52.3%) than on BS5A.2(T2) 19-3P1. The weight gain by the larvae (3.9 mg larva^{-1}) on *Bt* transgenic plants was significantly lower as compared to that on Semsen (12.7 mg larva^{-1}) and ICC 506 EB (11.2 mg larva^{-1}). The weight gain by *H. armigera* larvae on other transgenic lines ranged from 5.1 to 8.7 mg larva^{-1}, with significantly greater weight gain on BS5A.2(T2) 19-3P2 (8.7 mg larva^{-1}).

The transgenic line BS5A.1(T2) 18-1P1 recorded significantly lower leaf damage rating (DR: 2.2), followed by BS5A.1(T2) 18-2P1 (DR: 2.5), BS5A.2(T2) 19-1P2 (DR: 3.2), BS5A.2(T2) 19-2P1 (DR: 3.7), BS5A.2(T2) 19-3P1 (DR: 3.7) and BS5A.2(T2) 19-3P2 (DR: 4.3) as compared to Semsen (DR: 7.7) and ICC 506 EB (DR: 5.5) during 2012-13. The Larval survival on BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-2P1 was significantly lower (37.6%) as compared to that on ICC 506EB (79.3%) and Semsen (70.2%). Larval survival on other transgenic lines ranged from 40.1 to 48.1%. Weight gain by the *H. armigera* larvae was significantly lower on BS5A.1(T2) 18-1P1 (2.9 mg

Table 1. Evaluation of transgenic chickpeas for resistance to *H. armigera* under greenhouse conditions using cage technique

Genotype	2011-2012			2012-2013		
	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)
BS5A.1(T2) 18-1 P1	2.5^{ab}	52.3^{b}(46.5)	3.9^{a}	2.2^{a}	37.6^{a}(37.5)	2.9^{a}
BS5A.1(T2) 18-2 P1	4.1^{b}	49.7^{b}(44.8)	5.1^{ab}	2.5^{a}	41.2^{a}(39.8)	3.6^{a}
BS5A.2(T2) 19-1 P2	4.4^{b}	36.4^{a}(37.1)	5.2^{ab}	3.2^{ab}	40.1^{a}(39.2)	4.4^{ab}
BS5A.2(T2) 19-2 P1	1.6^{a}	41.3^{ab}(40.0)	6.4^{bc}	3.7^{ab}	37.6^{a}(37.8)	4.3^{ab}
BS5A.2(T2) 19-3 P1	2.8^{ab}	35.0^{a}(36.2)	7.3^{cd}	3.7^{ab}	41.1^{a}(39.9)	4.4^{ab}
BS5A.2(T2) 19-3 P2	4.3^{b}	50.8^{b}(45.4)	8.7^{d}	4.3^{bc}	48.1^{a}(43.9)	6.4^{b}
Semsen (Control)	7.8^{c}	75.7^{c}(60.5)	12.7^{e}	7.7^{d}	70.2^{b}(56.9)	13.6^{c}
ICC 506 EB (Resistant check)	8.0^{c}	72.3^{c}(58.2)	11.2^{e}	5.5^{c}	79.3^{b}(62.9)	17.0^{d}
Mean	4.4	51.7	7.5	4.1	49.4	7.1
SE +	0.5	3.5	0.5	0.4	5.9	0.7
Fp	<0.001	<0.001	<0.001	<0.001	0.009	<0.001
Vr	19.2	18.0	30.0	16.7	7.4	51.9
LSD (P 0.05)	1.7*	11.9*	1.8*	1.4*	19.8*	2.4*
CV (%)	16.9	9.8	10.5	14.9	17	14.5

*Figures followed by the same letter within a column are not significantly different at P<0.05
Figures in parenthesis are Angular transformed values.
HDR[1]- Leaf damage rating (1= <10 %, and 9= >80 % leaf area damaged)

larva^{-1}) as compared to ICC 506 EB (17.0 mg larva^{-1}) and Semsen (13.6 mg larva^{-1}) (Table 1).

Grain yield of transgenic chickpea lines under infested conditions

During 2011-12, there were significant differences in dry matter, pod weight, seed weight and the seed set between the transgenic and non-transgenic chickpea lines when infested with *H. armigera* larvae for 10 days. The weight of plant dry matter (5.0 to 6.5 g/3 plants) was significantly greater in BS5A.2(T2) 19-1P2 (6.5 g/3 plants) than Semsen (3.3 g/3 plants) and ICC 506 EB (3.5 g/3 plants). The pod weight was also significantly greater in BS5A.2(T2) 19-2P1 (2.6 g/3 plants), followed by BS5A.2(T2) 19-3P1 (2.3 g/3 plants), BS5A.2(T2) 19-3P2 (1.8 g/3 plants), BS5A.1 (T2) 18-1P1 (1.7 g/3 plants), BS5A.2(T2) 19-1P2 (1.6 g/3 plants), BS5A.1(T2) 18-2P1 (1.5 g/3 plants) and ICC506 EB (1.3 g/3 plants) than Semsen (0.6 g/3 plants). Higher seed weight was recorded on BS5A.2(T2) 19-3P1 (2.1 g/3 plants) and BS5A.2(T2) 19-2P1 (2.0 g/3 plants) compared to Semsen (0.5 g/3 plants) and ICC 506 EB (0.9 g/3 plants). The seed set in transgenic plants was higher than on non-transgenic plants. The number of seeds formed in BS5A.1(T2) 18-1P1 (16) and BS5A.1(T2) 18-2P1 (14) were significantly more as compared to that on Semsen (2) and ICC 506 EB (7) (Table 2).

During 2012-13, significantly higher dry matter weight was recorded in BS5A.2(T2) 19-2P1 (6.8 g/3 plants), and BS5A.1(T2) 18-2P1 (6.7 g/3 plants), BS5A.2(T2) 19-3P1 (6.7 g/3 plants), BS5A.2(T2) 19-3P2 (6.5 g/3 plants), BS5A.1(T2) 18-1P1 (6.2 g/3 plants) and BS5A.2(T2) 19-1P2 (5.2 g/3 plants) than in non-transgenic Semsen (3.6 g/3 plants) and ICC 506 EB (4.0 g/3 plants). The pod weight was significantly higher in BS5A.2(T2) 19-2P1 (4.1 g/3 plants) as compared to that on ICC 506 EB (1.2 g/

3 plants) and Semsen (1.3 g/3 plants). The seed weight was significantly higher in BS5A.2(T2) 19-2P1 (3.5 g/3 plants) as compared to Semsen (0.9 g/3 plants) and ICC 506 EB (1.0 g/3 plants). Similarly, number of seeds formed in BS5A.2(T2) 19-2P1 (26) were more compared to Semsen (3) and ICC 506 EB (6) (Table 2).

Significant differences in grain yield were observed between transgenic and non-transgenic plants infested with *H. armigera.* Since leaf damage was low in transgenic chickpea plants, the dry matter weight, pod weight, seed weight and number of seeds formed were significantly higher than on non-transgenic chickpea plants. In both the seasons, non-transgenic chickpeas yielded significantly lower compared to transgenic chickpeas. During 2012-13 planting, BS5A.2(T2) 19-2P1 had the highest dry matter weight (6.8 g/3 plants), pod weight (4.1 g/3 plants), seed weight (3.5 g/3 plants) and number of seeds formed (26) as compared to the other transgenic and non-transgenic chickpea lines.

Grain yield of transgenic and non-transgenic lines under un-infested conditions

In un-infested plants of transgenic and non-transgenic chickpeas during 2011-12, the dry matter weight was significantly higher in Semsen (9.3 g/3 plants) as compared to BS5A.1(T2) 18-2P1 (4.2 g/3 plants) and the dry matter weight in transgenic chickpeas ranged from 4.2 to 6.4 g/3 plants. The pod weight was significantly greater in BS5A.2(T2) 19-2P1 (3.3 g/3 plants), BS5A.2(T2) 19-1P2 (3.3 g/3 plants), BS5A.2(T2) 19-3P1 (3.0 g/3 plants), BS5A.1(T2) 18-2P1 (2.7 g/3 plants), BS5A.1(T2) 18-1P1 (2.6 g/3 plants), ICC 506 EB (2.4 g/3 plants) and BS5A.2(T2) 19-3P2 (2.2 g/3 plants) as compared to Semsen (1.0 g/3) (Table 3).

Seed weight was maximum in BS5A.2(T2) 19-2P1 (2.6 g/3 plants) and minimum in Semsen (0.9 g/3 plants). In other transgenic plants, the seed weight ranged between 2.3-2.4 g/3 plants. The number of seeds formed (3 plants^{-1}) was highest in

Table 2. Agronomic performance of transgenic chickpea lines (g/3 plants) resistant to *Helicoverpa armigera* under greenhouse condition using cage technique

Genotype	2011-2012				2012-2013			
	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds
BS5A.1(T2) 18-1 P1	5.8^{bc}	1.7^{b}	1.2^{bc}	16^{c}	6.2^{b}	2.2^{ab}	1.9^{ab}	21^{bcd}
BS5A.1(T2) 18-2 P1	6.0^{c}	1.5^{b}	1.4^{c}	14^{c}	6.7^{b}	2.0^{a}	1.9^{ab}	16^{b}
BS5A.2(T2) 19-1 P2	6.5^{c}	1.6^{b}	1.3^{bc}	10^{b}	5.2^{ab}	3.2^{bc}	2.9^{bc}	23^{cd}
BS5A.2(T2) 19-2 P1	5.0^{b}	2.6^{c}	2.0^{d}	9^{b}	6.8^{b}	4.1^{c}	3.5^{c}	26^{d}
BS5A.2(T2) 19-3 P1	6.4^{c}	2.3^{c}	2.1^{d}	10^{b}	6.7^{b}	3.2^{bc}	2.9^{bc}	19^{bc}
BS5A.2(T2) 19-3 P2	5.1^{b}	1.8^{b}	1.6^{c}	8^{b}	6.5^{b}	1.5^{a}	1.2^{a}	15^{b}
Semsen (Control)	3.3^{a}	0.6^{a}	0.5^{a}	2^{a}	3.6^{a}	1.3^{a}	0.9^{a}	3^{a}
ICC 506 EB (Resistant check)	3.5^{a}	1.3^{b}	0.9^{ab}	7^{b}	4.0^{a}	1.2^{a}	1.0^{a}	6^{a}
Mean	5.2	1.7	1.4	0.0	5.	2.3	2.0	0.0
SE +	0.0	0.0	0.1	0.0	0.5	0.3	0.3	0.0
Fp	<0.001	<0.001	<0.001	<0.001	0.014	0.004	0.006	<0.001
Vr	26.7	18.6	15.6	16.0	6.2	9.7	8.2	21.4
LSD (P 0.05)	0.7*	0.4*	0.4*	3.5*	1.7*	1.1*	1.1*	0.0*
CV (%)	6.4	11.3	12.9	16.2	12.9	19.9	23.4	15.3

*Figures followed by the same letter within a column are not significantly different at P<0.05.

Table 3 Agronomic performance of transgenic chickpea lines in un-infested plants (g/3 plants) under green house conditions

Genotype	2011-2012				2012-2013			
	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds
BS5A.1(T2) 18-1 P1	4.6^{a}	2.6^{bc}	2.3^{c}	21^{c}	5.4^{ab}	2.9^{b}	2.3^{b}	38^{b}
BS5A.1(T2) 18-2 P1	4.2^{a}	2.7^{bc}	2.3^{c}	23^{c}	4.2^{a}	3.3^{b}	2.9^{bc}	47^{cd}
BS5A.2(T2) 19-1 P2	5.6^{b}	3.3^{c}	2.3^{c}	38^{e}	5.4^{ab}	3.3^{b}	3.7^{d}	53^{d}
BS5A.2(T2) 19-2 P1	6.1^{bc}	3.3^{c}	2.6^{c}	43^{f}	6.1^{b}	5.2^{d}	5.0^{f}	64^{e}
BS5A.2(T2) 19-3 P1	6.4^{c}	3.0^{c}	2.4^{c}	28^{d}	5.7^{ab}	3.1^{b}	2.7^{b}	39^{bc}
BS5A.2(T2) 19-3 P2	6.2^{bc}	2.2^{b}	2.0^{c}	20^{bc}	5.7^{ab}	4.4^{cd}	4.6^{e}	53^{d}
Semsen (Control)	9.3^{d}	1.0^{a}	0.9^{a}	2^{a}	8.5^{c}	3.5^{a}	2.0^{a}	6^{a}
ICC 506 EB (Resistant check)	5.7^{b}	2.4^{bc}	1.5^{b}	16^{b}	5.7^{ab}	3.7^{bc}	3.6^{cd}	44^{bcd}
Mean	6.0	2.5	2.0	23.6	5.8	3.3	3.2	0.0
SE +	0.2	0.2	0.2	0.0	0.4	0.2	0.2	0.0
Fp	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
Vr	58.5	17.2	17.1	86.2	6.6	33.8	49.7	42.5
LSD (P 0.05)	0.6*	0.8*	0.2*	0.0*	1.5*	0.8*	0.6*	0.0*
CV (%)	4.7	13.9	14.2	8.1	11.4	10.4	9.0	8.8

*Figures followed by the same letter within a column are not significantly different at P<0.05.

BS5A.2(T2) 19-2P1 andlowest in Semsen (2). In other transgenic and non-transgenic plants, the seeds formed ranged from 16 to 43 (Table 3).

During 2012-13, similar trend was observed in dry matter weight, which was significantly higher in Semsen (8.5 g/3 plants) than in BS5A.1 (T2) 18-2P1 (4.2 g/3 plants). In other transgenic plants, the dry matter weight ranged from 4.2 to 6.1 g/3 plants. Pod weight was significantly higher in BS5A.2(T2) 19-2P1 (5.2 g/3 plants) as compared to Semsen (3.5 g/3 plants) and ICC 506 EB (3.7 g/3 plants), while in other transgenic plants, the pod weight ranged from 2.9 to 5.2 g/3 plants. Among transgenic plants, the seed weight was highest in BS5A.2(T2) 19-2P1 (5.0 g/3 plants) and lowest in BS5A.1(T2) 18-1P1 (2.3 g/3 plants). Whereas in non-transgenics, the seed weight was 6.0 g/3 plants in Semsen and 3.6 g/3 plants in ICC 506EB. Maximum number of seeds were formed in BS5A.2(T2) 19-2P1 (64), followed by BS5A.2(T2) 19-1P2 (53), BS5A.2(T2) 19-3P2 (53), BS5A.1(T2) 18-2P1 (47), ICC 506 EB (44), and BS5A.1(T2) 18-1P1 (38). Minimum seeds were formed in Semsen (6) (Table 3).

DISCUSSION

The present results confirmed the observations made by Acharjee *et al.* (2010), who reported significantly greater larval mortality of the *H. armigera* larvae fed on transgenic leaves (BS2A, BS5A and BS6H) than the larvae fed on control (Semsen and ICCV89314). Mogali *et al.* (2012) reported significantly lower leaf damage on *Bt* cotton leaves due to feeding by *H. armigera*as compared to the wild type. There was a significant increase in final body weight of the larvae fed on -ve control (111.5%) as compared to the larvae fed on transgenic plants (56.3%).

Similar observations on lower consumption of *Bt* cotton leaves by *H. armigera* larvae and higher mortality in choice tests has been reported by Zhang *et al.* (2004). Cotton bollworms fed on *Bt* cotton grew slower than those fed on non-*Bt* cotton, and also recorded less damage on transgenic *Bt* cotton

plants (Shudong *et al*., 2003). The larval population was significantly lower on the transgenic hybrids as compared to the non-transgenic commercial cultivars of cotton (Sharma and Pampathy, 2006).

The transgenic lines suffered lower leaf damage, reduced larval survival and weight gain by the *H. armigera* larvae as compared to non-transgenic chickpeas across the seasons as well as in different plantings under laboratory and glasshouse conditions. There was a significant difference in agronomic performance between transgenic and non-transgenic chickpea lines. In both the seasons, non-transgenic chickpeas yielded significantly lower compared to transgenic chickpeas.

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Comparative parasitisation of *Helicoverpa armigera* (Hubner) [Lepidoptera: Noctuidae] by *Campoletis chlorideae* Uchida [Hymenoptera: Ichneumonidae] on some chickpea varieties

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ABSTRACT: Chickpea varieties, commonly recommended for cultivation in Rajasthan, were screened for their preference by the pod borer, *Helicoverpa armigera* (Hubner), under natural infestation and the parasitisation efficacy of the larval parasitoid, *Campoletis chlorideae* Uchida. The variety Pratap Chana was most preferred by the pod borer, as it harboured the maximum numbers of eggs (15.85), larvae (19.05) and damaged pods (41.44); whereas, variety GNG 1581 was least preferred for egg laying (4.79); GNG 663 had lowest larval population (5.50); and RSG 888 had lowest numbers of damaged pods (4.19). The larval parasitoid, *C. chlorideae* was active from 15thDecember, 2014 to 26th January, 2015; but, the maximum parasitisation varied on different varieties. The observed abundance of the parasitoid, *C. chlorideae* was significantly more (10.47 per 4-m row) on chickpea variety Pratap Chana, while observed parasitisation (34.84%) was more on variety GNG 663. The coefficient of correlation between pod borer and its parasitoid was significant (r = +0.83) only for chickpea variety GNG 1581. The prevailing abiotic factors of the environment did not evince any significant effect on the population of pod borer and its larval parasitoid.

KEY WORDS: Chickpea, *Helicoverpa armigera*, parasitisation, *Campoletis chlorideae*

INTRODUCTION

Chickpea (*Cicer arietinum* L.), also known as Bengal gram, gram or *chana* is an important *rabi* pulse crop of India and is infested by several species of insects and other arthropods; however, the major pest of chickpea is the gram pod borer, *Helicoverpa armigera* (Hubner), which is a polyphagous, multivoltine and cosmopolitan pest, known to feed on 182 species of plants belonging to 47 families in India (Sithanantham, 1987 and Panwar, 1998). High polyphagy, mobility, reproductive rate and diapause are major factors contributing to its serious pest status (Fitt, 1989 and Sharma *et al.,* 2005). Over 250 natural enemies have been recorded on *H. armigera* (Romeis and Shanower, 1996) in different agro-ecosystems, however, the activity and abundance of natural enemies varies across crops (Pawar *et al.,* 1986), and different genotypes of the same crop (Romeis and Shanower, 1996; Sharma *et al.,* 2003; Dhillon and Sharma, 2007). Host plant selection by the female parasitoids, involves a series of complex responses in a non-random manner to a hierarchy of physical and/or

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chemical stimuli that lead them to their potential hosts (Vet and Groenewold, 1990; Lewis *et al.*, 1991; Tumlinson *et al.*, 1993). Parasitoids also respond to the volatiles emanating from both undamaged (McAuslane *et al.*, 1990; Li *et al.*, 1992; Turlings and Tumlinson, 1992; Udayagiri and Jones, 1992) and damaged (Whitman, 1988; Turlings *et al.*, 1990, 1995; Mattiaci *et al.*, 1994; de Moraes *et al.*, 1998; War *et al.*, 2011) plants. Genotypic resistance has a considerable influence on parasitism of insect pests in different crops. The nature of influence depends on the insect pest, natural enemy, and the crop (Sharma *et al.*, 2003). In chickpea, parasitisation of *H. armigera* larvae by *C. chlorideae* ranged from 8.33 to 28.00 per cent (Gupta and Raj, 2003), and varied considerably across genotypes (Kaur *et al.*, 2004); however, there is no information on genotypic effects on the activity and abundance of natural enemies in chickpea.

MATERIALS AND METHODS

A field experiment was conducted at the Instructional Farm, Rajasthan College of Agriculture, MPUAT, Udaipur, during *rabi* 2014-15. Six varieties recommended for the zone Pratap Chana, RSG-902, GNG-469, GNG-663, GNG-1581, RSG-888 were evaluated for their preference by the gram pod borer. The experiment was laid out in RBD with six treatments and four replications in plots of size 4m x 3m; planting the seed on 4th November, 2014 maintaining a spacing of 30cm x 10cm. All recommended agronomic practices including hoeing, weeding and irrigation were performed as and when needed following the package of practices for cultivation of chickpea.

During early hours of the day (7 to 9 am) observations on the number of eggs per plant as an evidence of preference for egg laying by *H. armigera* on the different gram varieties was recorded from 5 plants selected at random and tagged replicate-wise in each treatment (variety). Record of the total numbers of plants with egg laying per replicate for each variety screened was made and expressed as a percentage of plants harbouring *H. armigera* eggs in the different varieties for comparison. Similarly, observations for *H. armigera* larvae infesting the crop were taken along the 4-metre-row, selecting 3 rows from each plot/ replicate for each variety. From the same rows observed for the pest, the numbers of parasitized larvae were field collected and brought to the laboratory. Particular care was taken to record the influence of variety on parasitoid abundance and efficacy, for which, the field-collected parasitized larvae were maintained in glass jars of 500ml capacity separately until adult parasitoid emergence. The glass jars were covered with muslin cloth and fastened with rubber bands. The parasitoids were preserved for further study. The effective parasitisation (%) was computed using the methodology adopted by Tian *et al* (2008):

$$\text{Effective Parasitisation (\%)} = \frac{\text{Number of larvae parasitized}}{\text{Number of larvae effectively parasitized} + \text{Number of healthy larvae}} \times 100$$

Morphological characterization of the parasitoid was done using photographs of significant taxonomical characters taken under the stereozoom binoculars Stemi 2000 C of Carl Zeiss make. Necessary line drawings at a magnification of 7-X, for clarity, were drawn with the help of a drawing tube under the stereozoom binoculars Nikon SMZ 1500. The parasitoids collected were identified using standard references and internet sources (NBAIR, Bangalore).

RESULTS

From the Table (1) it can be observed that variety Pratap Chana was the most preferred variety of the pod borer as, on this variety, significantly the maximum mean numbers of eggs were laid (15.85 eggs per 4-m row), the maximum mean numbers of larvae were recorded (19.05 caterpillars per 4-m row) and the maximum damage to pods was also observed (41.44 pods per 4-m row). On the other hand, variety GNG 1581 happened to be the

Table 1. Screening of chickpea varieties against the gram pod borer during *rabi*, 2014-15

Gram Varieties	Egg-laying preference by pod borer (Mean ¹/row	Larval population of pod borer (Mean ¹/row)	Pod borer damaged Pods (Mean ¹/row)	Yield (Kg/plot) [Plot 12m²]	Yield (Kg/ha)
Pratap Chana	1.20^{f} {15.85}	1.08^{d} {19.05}	41.44^{d}	2.39^{ab}	1993.54
RSG 902	0.92^{b} {8.32}	0.73^{c} {8.32}	15.19^{c}	2.96^{b}	2463.75
GNG 469	1.10^{e} {12.60}	0.66^{b} {7.24}	9.64^{b}	2.20^{ab}	1834.79
GNG 663	1.07^{d} {11.75}	0.59^{a} {5.50}	5.81^{a}	2.41^{a}	2010.42
GNG 1581	0.68^{a} {4.79}	0.58^{a} {5.75}	5.74^{a}	1.75^{a}	1460.63
RSG 888	1.03^{c} {10.72}	0.73^{c} {7.94}	4.19^{a}	1.78^{a}	1480.00
S. Em. +	0.006	0.016	0.854	0.254	——
C.D. (5%)	0.018	0.049	2.572	0.765	——

Note: *Figures in* {} *are retransformed antilog values*

least preferred variety, as it harboured significantly the lowest numbers of eggs (4.79 eggs per 4-m row), lesser numbers of larvae (5.75 caterpillars per 4-m row) and also lower numbers of damaged pods (5.74 pods per 4-m row), however, variety GNG 663 harboured the least numbers of larvae (5.50 caterpillars per 4-m row) and variety RSG 888 had the lowest numbers of damaged pods (4.19 pods per 4-m row). When the yield parameters obtained from $12m^2$ plots were compared, the lowest yield was recorded for variety GNG 1581 (1.75 kg/plot), though it was least preferred by the pod borer, being at par with that of Pratap Chana, GNG 469, GNG 663 and RSG 888. The variety RSG 902 significantly yielded the maximum (2.96 kg/plot). Based on the yield attributes the varieties RSG 902, GNG 663, Pratap Chana and GNG 469 yielded relatively more than varieties GNG 1581 and RSG 888.

Natural parasitisation of *H. armigera* by the Ichneumonid parasitoid, *C. chlorideae* (Table: 2) indicated that parasitisation was significantly more on varieties GNG 663 (34.84 %), GNG 469 (33.16 %) and RSG 902 (30.27%); however, the numerical abundance of the parasitoid was significantly more on the variety Pratap Chana in terms of numbers (10.47) and mean parasite count (17.93). On the different varieties, the mean numbers of caterpillars in a 4-m row ranged from 5.50 (GNG 663) to 19.05 (Pratap Chana); the observed parasitoid abundance ranged from 2.74 (GNG 1581) to 10.47 (Pratap Chana); per cent parasitisation ranged from 24.74 (RSG 888) to 34.84 (GNG 663); and the mean parasite count ranged from 4.43 (GNG 1581) to 17.93 (Pratap Chana). From the Table (3) it is conspicuous that the effective parasitisation, as per method suggested by Tian *et al* (2008), was the maximum on variety GNG 469 (65.15 %), followed by that on Pratap Chana (61.40 %), while it was the minimum on RSG 888 (44.10 %). The seasonal parasitisation trend as given in Table (4) shows that irrespective of chickpea variety, natural field parasitisation was noted from 15th December, 2014 onwards that gradually increased in the subsequent weeks with a significant variation continuing up to the last week of January, 2015. The per cent parasitisation evaluated in the different varieties ranged from 18.97 to 32.63 for Pratap Chana; 20.30 to 46.40 for RSG 902; 20.70 to 41. 44 for GNG

Table 2. Natural parasitisation of *H. armigera* on different gram varieties during *rabi*, 2014-15

Gram Varieties	Larval population of pod borer (Mean No/row)	Observed Parasitoid* *C. chloridae*		Mean parasite count (No)
		Abundance (No)	Parasitization (%)	
Pratap Chana	1.08^{d}{19.05}	3.31^{c}[10.47]	31.26^{ab}(26.93)	1.2536^{b}{17.93}
RSG 902	0.73^{c}{8.32}	2.27^{ab}[4.65]	33.38^{abc}(30.27)	0.9340^{ab}{8.59}
GNG 469	0.66^{b}{7.24}	2.52^{b}[5.85]	35.16^{bc}(33.16)	1.0251^{ab}{10.59}
GNG 663	0.59^{a}{5.50}	1.89^{a}[3.06]	36.18^{c}(34.84)	0.7213^{a}{5.26}
GNG 1581	0.58^{a}{5.75}	1.80^{a}[2.74]	31.58^{ab}(27.42)	0.6461^{a}{4.43}
RSG 888	0.73^{c}{7.94}	1.90^{a}[3.11]	29.83^{a}(24.74)	0.6609^{a}{4.58}
S. Em. +	0.016	0.200	1.393	0.135
C.D. (5%)	0.049	0.602	4.197	0.405

**Figures in* () *are retransformed per cent values; Figures in* [] *are retransformed square values*; *Figures in* {} *are retransformed antilog values*

*Parasitoid abundance is on the basis of 7 observations during the season; the pod borer, *H. armigera* was parasitized by *C. chloridae*

Table 3. Effective parasitisation of *H. armigera* by *C. chlorideae* on different chickpea varieties during 2014-15 (as per method of Tian *et al.*, 2008)

Dates of Observation	Chickpea Varieties/parasitisation			Chickpea Varieties/ parasitisation					
	Atm. Temp. (°C)	R. H. (%)	Sunshine (hrs)	Pratap Chana	RSG 902	GNG 469	GNG 663	GNG 1581	RSG 888
15/12/2014	16.01	54.30	7.10	18.26	15.09	27.76	25.00	32.45	18.95
22/12/2014	13.59	57.70	7.70	42.51	54.78	60.01	47.26	36.40	42.61
29/12/2014	14.54	53.00	8.70	60.61	60.02	79.54	52.26	44.44	53.33
05/01/2015	14.80	72.00	3.90	55.40	45.05	70.07	36.97	42.19	52.22
12/01/2015	17.20	54.00	8.70	67.07	43.76	67.34	38.76	63.29	58.57
19/01/2015	14.70	65.00	7.60	92.38	92.34	73.53	81.47	75.19	0.00
26/01/2015	16.20	73.00	4.02	93.60	92.59	77.82	89.09	87.98	82.99
Seasonal Mean	15.29	61.29	6.82	61.40	57.66	65.15	52.97	54.56	44.10
r - value for mean Temp. & parasitisation				0.13	-0.18	-0.16	-0.08	0.41	0.31
r- value for mean R. H. & parasitisation				0.55	0.55	0.41	0.55	0.52	0.27
r- value for mean S-shine & parasitisation				-0.21	-0.21	-0.13	-0.25	-0.26	-0.39

Table 4. Seasonal parasitisation trend of gram pod borer by *C.chloridae* on different varieties of chickpea

Dates of Obser-vation	Mean Abiotic Factors			Chickpea Varieties/ parasitisation					
	Mean Atm. Temp. (C)	Mean R. H. (%)	Mean Sunshine (hrs)	Pratap Chana	RSG 902	GNG 469	GNG 663	GNG 1581	RSG 888
01/12/2014	21.62	52.00	8.90	2.79	1.27	0.96	1.27	0.83	0.71
08/12/2014	18.86	48.60	8.60	4.71	3.00	2.31	1.85	1.60	2.96
15/12/2014	16.01	54.30	7.10	7.83 (18.97)	2.81 (20.30)	2.60 (26.27)	1.50 (26.44)	2.60 (28.25)	3.21 (21.92)
22/12/2014	13.59	57.70	7.70	4.40 (28.90)	2.27 (39.27)	1.83 (38.50)	1.40 (40.12)	1.75 (32.04)	2.02 (29.11)
29/12/2014	14.54	53.00	8.70	3.25 (32.63)	0.83 (30.55)	0.71 (41.44)	0.69 (34.05)	0.31 (21.03)	1.31 (29.28)
05/01/2015	14.80	72.00	3.90	2.42 (28.00)	0.92 (24.31)	0.75 (33.24)	0.85 (27.40)	0.69 (22.07)	0.92 (28.47)
12/01/2015	17.20	54.00	8.70	1.23 (26.13)	0.64 (22.42)	0.73 (32.71)	0.79 (29.48)	0.44 (27.57)	0.71 (26.97)
19/01/2015	14.70	65.00	7.60	0.17 (29.06)	0.21 (46.40)	0.27 (30.87)	0.23 (45.90)	0.17 (28.47)	0.21 (12.86)
26/01/2015	16.20	73.00	4.02	0.10 (25.53)	0.06 (30.87)	0.14 (20.70)	0.12 (41.71)	0.10 (33.33)	0.10 (26.44)

* Figures in parentheses are percent values of parasitisation

469; 26.44 to 45.90 for GNG 663; 21.03 to 33.33 for GNG 1581 and 12.86 to 29.28 for RSG 888.

The abiotic factors of the environment did not significantly affect the effective parasitisation (%) of *H. armigera* by *C. chlorideae*; however, atmospheric temperature had a variable response among the chickpea varieties; relative humidity was uniformly positively correlated to parasitisation across the varieties and sunshine showed a negative correlation with parasitisation for all the varieties evaluated (Table: 3). The observed numerical abundance of the larval parasitoid of the pod borer showed significant negative correlation with the mean atmospheric temperature only on chickpea variety RSG 902 ($r = - 0.78^*$); while, on other varieties the correlation coefficients for different factors of the environment had no significant relationship. Likewise, the population of *H. armigera* had a negative correlation with the mean relative humidity that was significant only on chickpea variety GNG 663 ($r = - 0.71^*$). The relationship between pod borer and its parasitoid evinced a significant positive correlation ($r = 0.83^*$) only on the chickpea variety GNG 1581 (Table: 5).

The larval parasitoid of *H. armigera* was identified as *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) with the help of identification key provided by NBAIR, Bangalore (URL: www.nbair.res.in, 2013) and has been presented in Plate I along with the life stages of the pod borer, *Helicoverpa armigera* (Hubner). As per key, the important taxonomic features observed for the species include: areolet in forewing receiving second recurrent vein a little before middle and the

Table 5. Impact of abiotic/ biotic factors on the gram pod borer and its parasitoid on different gram varieties

Dates of Observation	Mean Abiotic Factors			Population of the pod borer, *H. armigera* and larval parasitoid, *C. chloridae* (1/row)											
	Atm. Temp. (°C)	R. H. (%)	Sun-shine (hrs)	Pratap Chana		RSG 902		GNG 469		GNG 663		GNG 1581		RSG 888	
				H	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**
01/12/14	21.62	52.00	8.90	2.79	—	1.27	—	0.96		1.27	—	0.83	—	0.71	—
08/12/14	18.86	48.60	8.60	4.71	—	3.00	—	2.31		1.85	—	1.60	—	2.96	—
15/12/14	16.01	54.30	7.10	7.83	1.75	2.81	0.50	2.60	1.00	1.50	0.50	2.60	1.25	3.21	0.75
22/12/14	13.59	57.70	7.70	4.40	3.25	2.27	2.75	1.83	2.75	1.40	1.25	1.75	1.00	2.02	1.50
29/12/14	14.54	53.00	8.70	3.25	5.00	0.83	1.25	0.71	2.75	0.69	0.75	0.31	0.25	1.31	1.50
05/01/15	14.80	72.00	3.90	2.42	3.00	0.92	0.75	0.75	1.75	0.85	0.50	0.69	0.50	0.92	1.00
12/01/15	17.20	54.00	8.70	1.23	2.50	0.64	0.50	0.73	1.50	0.79	0.50	0.44	0.75	0.71	1.00
19/01/15	14.70	65.00	7.60	0.17	2.00	0.21	2.50	0.27	0.75	0.23	1.00	0.17	0.50	0.21	0.00
26/01/15	16.20	73.00	4.02	0.10	1.50	0.06	0.75	0.14	0.50	0.12	1.00	0.10	0.75	0.10	0.50
Correlation for Atm. Temp. with H/C				0.04	-0.52	0.17	**-0.78***	0.12	-0.60	0.37	-0.58	0.05	0.23	0.05	-0.32
Correlation for RH with H/C			-0.56	-0.43	-0.62	-0.03	-0.60	-0.49	**-0.71***	0.19	-0.46	-0.22	-0.59	-0.49	
Correlation for sunshine with H/C				0.26	0.42	0.33	0.30	0.31	0.43	0.44	0.03	0.18	-0.01	0.29	0.29
Correlation between pest and parasitoid				—	0.12	—	0.05	—	0.25	—	-0.21	—	**0.83***	—	0.43

* Indicates t-value being significant at P=0.05; H = *H. armigera* , C = *C. chloridae*

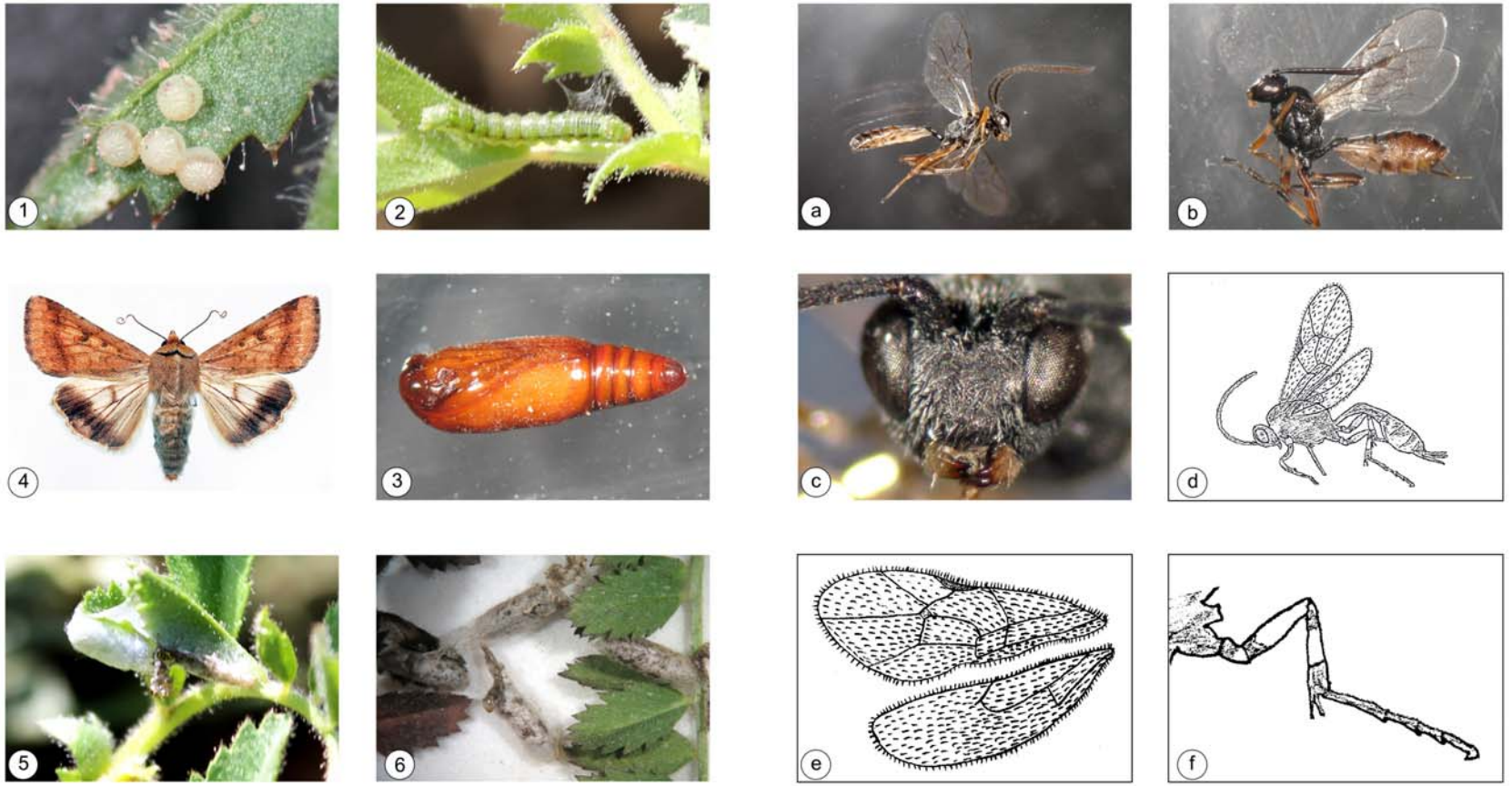

Plate: I Life cycle of *H. armigera* and morphological characterization of the larval parasitoid, *C. chlorideae*

1. Eggs; 2. Caterpillar; 3. Pupa; 4. Adult – *Helicoverpa armigera* (Hubner); 5. Field parasitization of *H. amigera*; 6. Cocoon of *C. chlorideae*

a. *C. chlorideae* (♂); b. *C. chlorideae* (♀); c. Clypeus with median tooth ; d. Female diagram lateral view; e. Areolet in forewing; f. Two segmented hind trochanter

apical margin of clypeus with an obtuse median tooth.

DISCUSSION

The entire collection of 362 parasitoids happened to be males and females of *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae). The overall assessment indicated that variety Pratap Chana was most preferred by the pod borer and the associated parasitoid was also the maximum on this variety; while, the variety GNG 1581 was least preferred by the pod borer and was also least visited by the parasitoid, defining the density-dependent activity of the parasitoid. Earlier, Ramegowda *et al* (2007) observed that of the 24 genotypes screened against *H. armigera*, ICC 506 (resistant control) and A1 (local control), BG-1039, P-1772 B, L-550 and 86019 had minimum ova load and were at par with ICC-506 and superior to A1, which recorded 2.70 ova per plant. Deshmukh *et al* (2010) reported chickpea genotypes BG-372, HC-1, SAKI-9516, Vijay and Avrodhito to be comparatively less susceptible as they harboured lower larval population (1.07 to 1.32 larvae/ plant) and had lower damage to pods (11.41 to 14.16%). Likewise, the mean larval population was lowest (<4.75 larvae/5 plants) on RSG-931 and GNG-1488, which were categorized as the least susceptible to the gram pod borer under hyper arid partial irrigated western plain zone of Rajasthan (Chandra and Nanda, 2013).

Earlier reports indicate that the egg parasitoid, *Trichogramma* spp. and the larval parasitoids, *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae), *Carcelia illota* Curran, *Palexotista* spp., and *Goniozus* spp. are predominant parasitoids of *H. armigera* in different agro-ecosystems. It has also been observed that the activity and abundance of natural enemies varies across crops (Pawar *et al.,* 1986), and different genotypes of the same crop (Romeis and Shanower, 1996; Sharma *et al.,* 2003; Dhillon and Sharma, 2007). In chickpea, parasitism of *H. armigera* larvae by *C. chlorideae* ranged from 8.33 to 28.00 per cent (Gupta and Raj, 2003), and varied considerably across genotypes (Kaur *et al.,* 2004). Studies were undertaken to identify pigeonpea, *Cajanus cajan* (L.) and the wild relative of pigeonpea, *Cajanus scarabaeoides* (L.) (Accession ICPW 125) genotypes that are hospitable to the pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and the larval parasitoid, *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) for the management of this pest in pigeonpea based cropping systems. Percentage parasitisation of *H. armigera* larvae by *C. chlorideae* females was greater under no-choice conditions than under multi-choice conditions because of forced parasitisation under no-choice conditions. Lowest parasitisation was recorded on the wild relative, ICPW 125, which may be due to long non-glandular hairs and low survival of *H. armigera* larvae. Parasitisation of *H. armigera* larvae was greater under no-choice, dual-choice and/or multi-choice conditions on ICPL 87, ICPL 87119 and ICPL 87091, which are susceptible to *H. armigera*, than on the pod borer-resistant genotypes ICPL 332WR, ICPL 84060 and ICPB 2042; while survival and development of the parasitoid was better on *H. armigera* larvae fed on ICPL 87, ICPL 87119, LRG 41, ICP 7035 and ICPL 87091 than on ICPL 332WR, ICPL 84060, ICPB 2042 and ICPW 125. The genotypes ICPL 87, ICPL 87119, LRG 42 and ICPL 87091 that are hospitable to *C. chloridae*, are better suited for use in integrated pest management to minimize the losses due to *H. armigera* in pigeonpea (Hugar *et al.,* 2014). It thus becomes increasingly clear that germplasm susceptible to pest attack happen to attract more parasitoids leading to higher parasitisation.

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Establishing biodiversity in dwarf honey bee, *Apis florea* F. (Hymenoptera: Apidae) workers from north western India based on morphometrics of antenna and mouth parts

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ABSTRACT: The present study was conducted on two head appendages viz. antenna and mouth parts to report morphometric differences in 7 different populations of *Apis florea* F. collected from foot hill regions of Himachal Pradesh (Bangana, Chintpurni, Hamirpur, Parwanoo, Daulatpur and Gagret) and Chandigarh plains (Chandigarh) of north western India. Collection was made during mid June- mid September months of the year, 2010-14. Ten morphometric characters and 4 biometric indices were statistically analyzed by means of factor analysis, discriminant analysis and cluster analysis. Chandigarh and Gagret regions having low altitude tend to show higher values of more number of morphometric characters while Bangana region with high altitude showed the opposite. For both antenna and tongue maximum numbers of characteristics with higher values were in Gagret region while maximum number of characteristics with lower values was exhibited by Bangana population of *A. florea*. The majority of characteristics of the tongue were significantly correlated with altitude suggesting that characteristics associated with foraging were more prone to be affected by altitude.

KEYWORDS: *Apis florea*, biodiversity, intra-specific, morphometrics, antenna, mouth parts

INTRODUCTION

Head of honey bee is triangular shaped bearing two compound eyes and three occelli located on the top of the head. Front side of the head bears the antenna and mouth parts. The two antennae are closely placed near the upper centre of the head. Each antenna consists of a single long joint connected to a prominent knob inserted into a socket. Each antenna has an elongated scape, a pivoted pedicel and a segmented flagellum (geniculate type), which is composed of ten flagellomeres in the worker (Winston, 1987) honey bees.

The mouthparts of dwarf honey bees are of chewing and lapping type. They consist of paired mandibles and the proboscis which is made of labium and maxillae. Proboscis is a more complicated structure and performs the major function of ingestion of liquid food. The role of glossa in pollen carriage was reported by Michener *et al.* (1978) since pollen grains were frequently trapped by glossal hair. Entire proboscis can be folded to Z shape when not in use. Michener and Brooks (1984) reported that flabellum at the tip of tongue helped in absorption and transportation of liquids to the mouth.

Goetze (1964) introduced biometric methods into the micro-systematics of honey bees. Using

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measurements of the body parts he introduced descriptions and classification of honey bees. There are many species specific quantitative characters especially length and width of tongue, antenna, forewing, hind wing, hind leg etc. of the generally recognized *Apis* species.

Morphometric studies on *A. florea* have been made throughout the world by scientists using different characters (Rinderer *et al.,* 1995). Although, there is extensive data related to general biology of *A. florea*, there is still a lack of sufficient morphometric data unlike Iran and Thailand. Morphometric study of dwarf honey bee from India has been reported for some selected characters only (Bhandari, 1983 and Sharma, 1983). In the present investigations, therefore, areas of north-western region of India were chosen to investigate the dwarf honey bee diversity on the basis of morphometric studies.

MATERIALS AND METHODS

Study area: Sample bees were collected from state of Himachal Pradesh and Chandigarh (UT). Detailed description of collection area is given in Table 1 and Fig 1 & 2.

Sample Collection, preparation and examination: Forager bees were collected from each region @150 bees / region. Experimental work was carried out in Entomology laboratory in Department of Zoology, Panjab University. Antenna and tongue were disarticulated and preserved in Pampell's fixative for further processing. Slides were prepared after washing in distilled water and specimens were mounted using a drop of Arabic gum. Slides were oven dried at 50°C for 2-4 hours. All measurements were made using stereo zoom microscope (Radical RSM-9) fitted with camera and provided with sofware (ProgResR CT5 USBC) and scale.

Sample and characteristics chosen

Ten morphometric characters studied included length and width of antennae and mouth parts and 4 biometric indices as considered by Ruttner *et al.* (1978) and Ruttner (1980, 1988). These are: Length of scape (SpL), Length of pedicel (PdL), Length of flagellum (FgL), Length of antenna (AtL), Length of postmentum (PoL), Breadth of prementum (PmB), Length of prementum (PmL), Length of glossa (GL), Length of labial palp (LpL) and Length of tongue (ToL) as shown in Figure 3 and 4.

Biometric indices

Antenna and tongue measurements of seven populations from different regions have been described with statistical analysis such as means, standard deviation and coefficient of variation of 4 biometric indices such as SpL / FgL, PmL / PmB, GL / PmL, LpL / PmL respectively termed as

Table 1 Localities, geographical coordinates and altitude from where *A. florea* bees were sampled

S. No.	Collection Area	Latitude	Longitude	Altitude
1	Chandigarh	30°43'59.93"N	76°46'45.90"E	365 m
2	Gagret	31°39'37.88"N	76°03'35.09"E	439 m
3	Daulatpur	31°46'58.52"N	75°59'23.51"E	521 m
4	Parwanoo	30°50'17.02"N	76°57'30.60"E	672 m
5	Hamirpur	31°41'10.23"N	76°31'16.71"E	785 m
6	Chintpurni	31°48'34.53"N	76°07'27.90"E	975 m
7	Bangana	31°39'39.69" N	76°20'51.44"E	1100 m

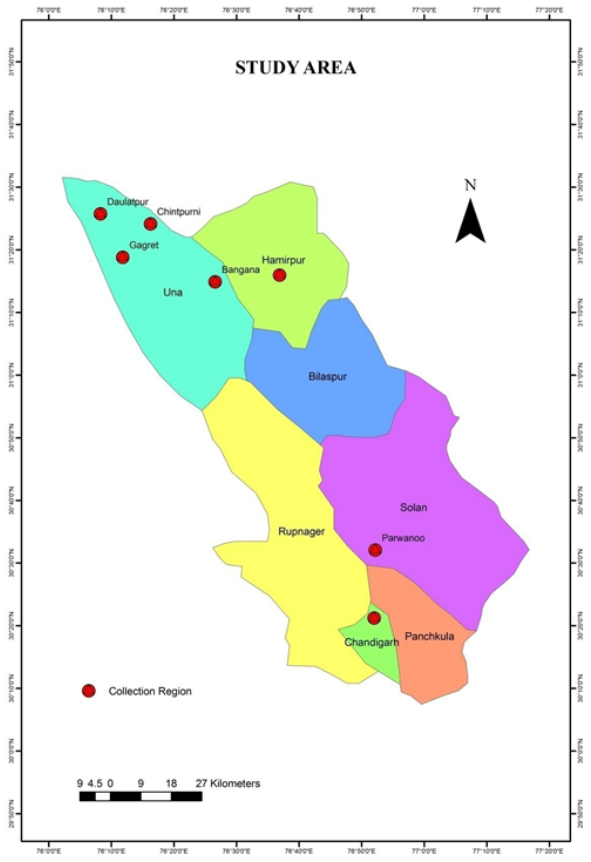

Figure 1 Map showing the locations from where the *Apis florea* sample were surveyed and collected

antenna- scapoflagellal index, Premental index, Glosso premental index and Labiopremental index.

Statistical analysis

A descriptive statistical analysis was carried out and comparisons between locations were determined by mean, standard deviation, analysis of variance (ANOVA), multiple range test, Coefficient of variation and correlation. Statistical analyses were performed using "SPSS" package.

We used 10 morphometric characters (length and width of tongue and antenna) obtained by morphometrics in Principle Component Analysis (PCA) in order to classify the populations of *Apis florea*, worker bees based on the distance from the control individuals. Morphometric analyses of populations were done using means, standard

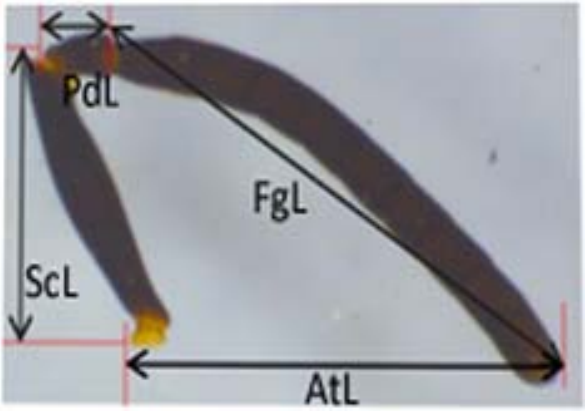

Figure 2 Morphometric characteristics of antenna of *Apis florea*.

deviations and covariances of the morphometric characters. We used one way ANOVA from the individual to the centroid of the group to compare each individual. Data were also analyzed with coefficient of variation and correlation to compare population. Bonferroni test was used to compare multivariate population means between groups. A cluster analysis was carried out of classify populations into morphocluster.

RESULTS

The data obtained by statistical analysis from 10 characteristics of antenna and tongue concerns differences in size *i.e.* length and width. The total variation in all populations of *Apis florea* is worth consideration in identification of races which is altitude specific. Length of antenna (total length) ranged from 2.753 to 2.813 mm and that of tongue (total length) from 3.117 to 3.211 mm. Our results showed that in populations of hilly regions the tongue was longer than plain region i.e. Chandigarh. This is in agreement with Allen's rule (Allen, 1877)

In comparison to other regions, the Chandigarh population exhibited significantly higher value ($p<0.01$) for scape length, pedicel length, flagellum length, and total tongue length; Gagret population for pedicel length, flagellum length, total antennal length and total tongue length; Daulatpur population for scape length, flagellum length and prementum width; Parwanoo population for pedicel length,

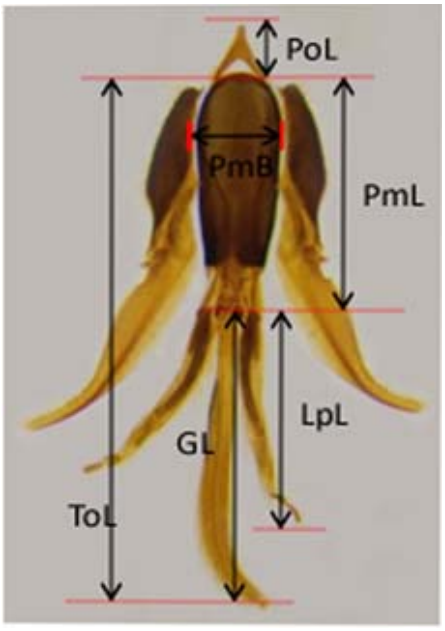

Figure 3 Morphometric characteristics of mouth parts of *Apis florea.*

flagellum length; Hamirpur population for pedicel length, flagellum length, total antennal length; Chintpurni population for pedicel length; Bangana population for flagellum length, prementum width (Table 2).

Antenna: The values were statistically significant ($p<0.05$) for scape length in Gagret, Parwanoo populations; pedicel length in Daulatpur and Bangana populations; flagellum length in Chintpurni and Bangana populations; total antennal length in Chandigarh, Daulatpur, Parwanoo, Chintpurni and Bangana populations; postmentum length in all populations, prementum width in Chandigarh, Gagret, Parwanoo, Hamirpur, and Chintpurni populations; prementum, glossa and labial palp length in all the populations of *A. florea*. The two morphometric characteristics with insignificant value were scape length in Hamirpur, Chintpurni and Bangana population, and tongue length in

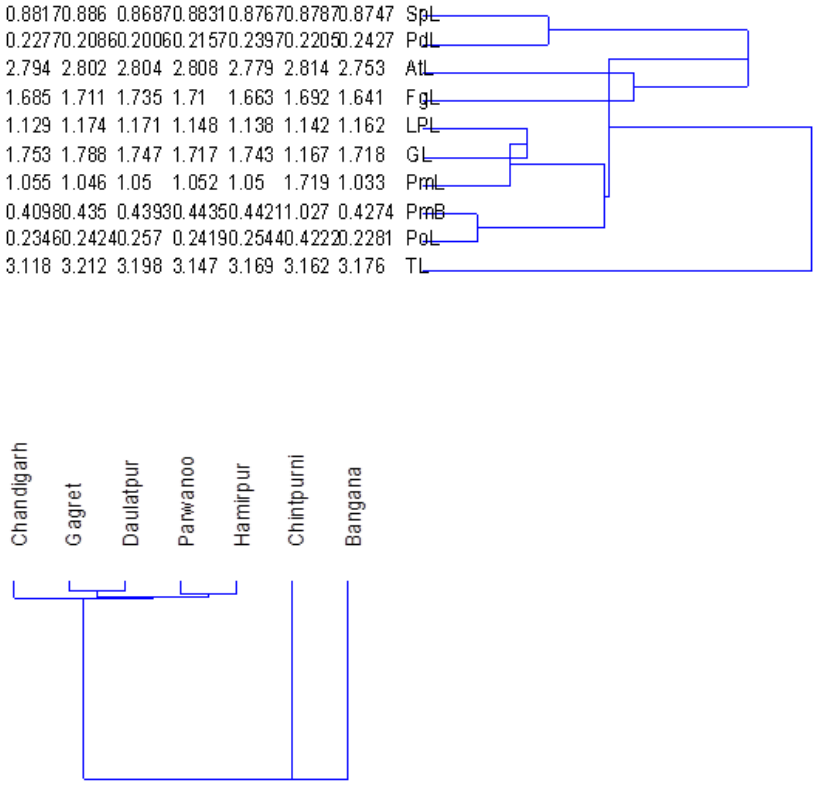

Figure 4 Cluster analysis (Paired group, Euclidean, two way)

Daulatpur, Parwanoo, Hamirpur, Chintpurni and Bangana populations of *A. florea*. Further, comparison of characteristics by ANOVA revealed that length of scape, pedicel, flagellum and total antennal length (F= 4.549, 39.345, 41.183 and 19.949 respectively and d.f. =6 (between groups), 133 (within groups), 139 (total), were significantly different P<0.001) in bee samples collected (Table 3).

Tongue: There were no significant differences between tongue segment such as postmentum (p>0.05), labial palp (p>0.05) and glossa (p>0.05) but there were significant differences in the total tongue length of *A. florea* (p<0.05) populations. Comparison of characteristics by ANOVA revealed that length of postmentum (F=910.897), breadth of prementum (F=7047.105), length of prementum (F=22617.056), glossa length (F=2138.839), labial palp length (F=1327.478) and total tongue length (F=56.948), d.f. = 6 (between groups), 133 (within groups), 139 (total), were significantly different (P<0.001) in bee samples collected (Table 3).

On the basis of the data observed from foothills of Himachal Pradesh and Chandigarh plains regions we conclude that single species of *Apis florea* with two morphoclusters persists throughout the study area (Figure 4). Common ecotypes are found in most of the regions due to the migratory tendency of the bee. For more precision and possibility of existence of ecotypes a wider range of geographic areas is needed.

DISCUSSION

The present study on *Apis florea* indicated that altitude variations affect the characteristic size of honey bee (Table 2 and 3). This study corresponded well with earlier work on *A. florea*. The characteristics of head appendages (antenna and tongue) with respect to length and width were affected by geographic location of the colony and showed significant differences (pd”0.01). The differences in scape, pedicel and flagellum length could be correlated to antenna length as total antenna length was significantly positively correlated to these segments. The differences in postmentum, prementum, labial palp, glossa length, could be correlated to total length of tongue.

The SpL in antenna of *A. florea* from different study regions was in following order: Gagret (0.886 mm)> Parwanoo (0.883 mm) > Chandigarh (0.881 mm) > Chintpurni (0.878 mm) > Hamirpur (0.876 mm) > Bangana (0.874 mm) respectively. Sharma (1983) reported length of scape in the following order, as Hamirpur (0.889 mm) > Una (0.886 mm) > Hoshiarpur (0.884 mm) > Kalka (0.883 mm) which is in corroboration of the present findings. However, the scape length of Hamirpur bees in the present case was distinctly less than that observed by Sharma (1983). The PdL in antenna of *A. florea* was in the order of : Bangana (0.242 mm) > Chandigarh (0.227 mm) > Chintpurni (0.221 mm) > Parwanoo (0.215 mm) > Gagret (0.208 mm) > Daulatpur (0.201 mm) respectively. This was the only antennal parameter that had highest value in the Bangana bees which otherwise showed the shortest antenna. Highest FgL of antenna was recorded for Daulatpur (1.734 mm) and lowest for Bangana (1.641 mm) populations. Bangana bees also had the shortest total length. Sharma (1983) reported flagellum length for *A. florea* from Una (1.743 mm), Kalka (1.736 mm), Hoshiarpur (1.703 mm) and Hamirpur (1.702 mm). Al-Kahtani and Taha (2014) observed significant differences in flagellum length of antenna for *A. florea* from Al-Ahsa (1.73 mm) and Jubail (1.69 mm) provinces respectively of Saudi Arabia. AtL in the present studies on *A. florea* was observed to range between 2.753 mm to 2.813 mm across seven regions of study and this difference was significant (pd”0.01). Sharma (1983) had observed similar variations in the populations of *A. florea* studied by him and reported that the total length of antenna was highest in *A. florea* from Una (2.846 mm) followed by Kalka (2.834 mm), Hamirpur (2.798 mm) and Hoshiarpur(2.784 mm). Al-Kahtani and Taha (2014) reported higher antennal length for dwarf honey bees from Al-Ahsa (2.75 mm) than those from Jubail (2.70 mm) provinces from Saudi Arabia. They (Al-Kahtani and Taha, 2014) reported significant correlation of body size with characteristics from head region i.e. antenna and mouth parts. These observations are in agreement

Table 2. Comparative morphometric data of Apis florea F. workers collected from 7 regions (Chandigarh and Himachal Pradesh). Means ± Standard deviation (in mm) of 10 morphological characters (mm) of worker bees.

Characters	Chandigarh (a)	Gagret (b)	Daulatpur (c)	Parwanoo (d)	Hamirpur (e)	Chintpurni (f)	Bangana (g)
1 SpL	0.881±0.005 c# 0.68%	0.886±0.004 c* 0.41%	0.868±0.005 a# b*d* 0.61%	0.883±0.004 c* 0.54%	0.876±0.003 Ns 2.64%	0.878±0.009 Ns 1.05%	0.874±0.007 Ns 2.04%
2 PdL	0.227±0.002 b*c*d# e# g* 1.18%	0.208±0.004 a*e*f# g* 1.84%	0.200±0.009 a*d*e*f*g* 5.57%	0.215±0.013 a# c*e*g* 6.28%	0.239±0.010 a# b*c*d*f* 4.58%	0.220±0.005 b# c*e*g* 2.39%	0.242±0.019 a*b*c*d*f* 8.05%
3 FgL	1.684±0.002 b*c*d# e*g* 0.16%	1.711±0.004 a*c# e*g* 0.23%	1.734±0.013 a*b# d*e*f*g* 0.76%	1.709±0.018 a# c*e*g* 1.11%	1.662±0.029 a# b*c*d*f*g# 2.37%	1.691±0.028 c*e*g* 1.70%	1.641±0.021 a*b*c*d*e# f* 1.33%
4 AtL	2.794±0.018 g* 0.29%	2.802±0.019 e# g* 0.33%	2.804±0.029 e*g* 0.69%	2.808±0.014 e*g* 0.52%	2.779±0.073 c# d*f*g* 1.36%	2.813±0.020 e*g* 0.73%	2.753±0.022 a*b*c*d*e*f* 0.81%
5 PoL	0.234±0.013 c*e*f* 1.43%	0.242±0.010 c*e*f*g* 4.40%	0.257±0.009 a*b*d*f*g* 3.74%	0.241±0.011 c*e*f*g* 4.58%	0.254±0.010 a*b*d*f*g* 4.08%	0.237±0.009 a*b*c*d*e*g* 2.68%	0.228±0.012 b*c*d*e*f* 5.28%
6 PmB	0.409±0.023 b*c*d*e*f*g* 5.75%	0.434±0.012 a*f* 0.63%	0.439±0.010 a*f*g# 2.46%	0.443±0.009 a*f*g* 2.08%	0.442±0.009 a*f*g* 0.95%	0.422±0.011 a*b*c*de*g* 0.93%	0.427±0.011 a*c#d*e*f* 2.69%
7 PmL	1.054±0.007 b*f*g* 0.75%	1.046±0.003 a*f*g* 0.35%	1.049±0.007 f*g* 0.72%	1.051±0.009 f*g* 0.90%	1.049±0.007 f*g* 0.75%	1.027±0.009 a*b*c*d*e*g* 0.46%	1.033±0.007 a*b*c*d*e*f* 0.68%
8 GL	1.753±0.004 b*d*f*g* 0.26%	1.788±0.013 a*c*d*e*f*g* 0.20%	1.746±0.021 b*d*f*g* 1.24%	1.717±0.014 a*b*c*e*f* 0.86%	1.742±0.018 b*d*f*g* 1.67%	1.718±0.009 a*b*c*d*e*g* 3.36%	1.717±0.007 a*b*c*e*f* 0.45%
9 LpL	1.129±0.022 f* 1.95%	1.174±0.036 f* 3.14%	1.171±0.028 f* 6.74%	1.147±0.034 f* 3.92%	1.138±0.027 f* 4.32%	1.166±0.029 a*b*c*d*e*g* 6.73%	1.162±0.024 f* 4.71%
10 ToL	3.117±0.005 b# 0.17%	3.211±0.005 a# 0.17%	3.197±0.027 ns 0.87%	3.147±0.027 ns 0.88%	3.168±0.017 Ns 0.58%	3.161±0.212 Ns 0.87%	3.176±0.022 Ns 0.71%

Values are expressed as Mean ± S.D. Lower case alphabet 'a' represents Chandigarh, 'b'=Gagret, 'c'= Daulatpur,'d'= Parwanoo, 'e' =Hamirpur, 'f'= Chintpurni and 'g'= Bangana.
Mean ± S.D. is followed by Post hoc test of Bonferroni to compare the values of different regions with each other in which Means are statistically highly significant at pd"0.01*, significant at pd"0.05# and non significant above 0.05 =ns.
It is followed by C.V. %
All the mean values are in mm.

with the present data. A significant positive correlation was found between length of antenna and flagellum (r=0.619) (Table 4). Postmentum length was having weak positive correlation with width (r=0.307) of prementum. Negative correlation was found for pedicel length with that of flagellum length (r=-0.665) and antennal length (r=-0.365, p<0.01).

Table 3. Multiple comparisons with ANOVA

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
SpL	Between Groups	0.004	6	0.001	4.549	<.001**
	Within Groups	0.02	133	0		
	Total	0.024	139			
PdL	Between Groups	0.029	6	0.005	39.345	<.001**
	Within Groups	0.016	133	0		
	Total	0.045	139			
FgL	Between Groups	0.12	6	0.02	41.183	<.001**
	Within Groups	0.065	133	0		
	Total	0.185	139			
AtL	Between Groups	0.053	6	0.009	19.949	<.001**
	Within Groups	0.058	133	0		
	Total	0.111	139			
PoL	Between Groups	0.563	6	0.094	910.897	0
	Within Groups	0.014	133	0		
	Total	0.576	139			
PmB	Between Groups	6.073	6	1.012	7047.105	0
	Within Groups	0.019	133	0		
	Total	6.092	139			
PmL	Between Groups	7.727	6	1.288	22617.056	0
	Within Groups	0.008	133	0		
	Total	7.735	139			
GL	Between Groups	5.792	6	0.965	2138.839	0
	Within Groups	0.06	133	0		
	Total	5.852	139			
LPL	Between Groups	69.151	6	11.525	1327.478	0
	Within Groups	1.155	133	0.009		
	Total	70.305	139			
TL	Between Groups	0.116	5	0.023	56.948	0
	Within Groups	0.046	114	0		
	Total	0.163	119			

The ToL was found to be highest in *A. florea* from Gagret (3.211 mm) followed by Bangana (3.176 mm), Hamirpur (3.168 mm), Chintpurni (3.161 mm), Parwanoo (3.147 mm) and Chandigarh (3.117 mm). Sharma (1983) reported highest tongue length for Hoshiarpur (3.270 mm) followed by Hamirpur (3.224 mm), Kalka (3.203 mm) and Una (3.200 mm). These values were higher than those recorded in the present study. Al-Kahtani and Taha (2014) reported higher tongue length for *A. florea* from Al-Ahsa (3.22 mm) than from Jubail (3.14 mm) province of Saudi Arabia. Rinderer (1995) reported the total tongue length for *A. florea* from Thailand as 3.273 mm. Wongsiri *et al*. (1996)

Table 4. Comparative biometric indices of antenna and tongue of *Apis florea* workers collected from seven regions

Characteristic	Chandigarh (a)	Gagret (b)	Daulatpur (c)	Parwanoo (d)	Hamirpur (e)	Chintpurni (f)	Bangana (g)
1 SpL/FgL	0.523±0.007 c* 0.66%	0.517±0.005 c*g* 0.46%	0.500±0.001 a*b*d*e*f*g* 1.07%	0.516±0.001 c*g* 1.47%	0.527±0.004 c* 4.06%	0.519±0.002 c*g* 1.99%	0.533±0.003 b*c*d*f* 2.75%
2 PmL/PmB	2.588±0.043 b*c*d*e*f*g* 7.44%	2.405±0.004 a*f* 0.75%	2.390±0.013 a*f* 2.51%	2.373±0.012 a*f* 2.42%	2.374±0.005 a*f* 0.96%	1.673±0.003 a*b*c*d*e*g* 1.07%	2.419±0.014 a*f* 2.77%
3 GL/PmL	1.662±0.003 b*d*f* 0.81%	1.709±0.001 a*c*d*e*f*g* 0.35%	1.664±0.006 b*d*f* 1.64%	1.632±0.004 a*b*c*f*g* 1.26%	1.659±0.006 b*d*f* 1.72%	1.678±0.004 a*b*c*d*e*g* 3.15%	1.662±0.002 b*d*f* 0.78%
4 LPL/PmL	1.070±0.004 f* 1.98%	1.122±0.007 f* 3.12%	1.115±0.017 f* 6.90%	1.091±0.010 f* 4.13%	1.084±0.010 f* 4.22%	1.239±0.027 a*b*c*d*e*g* 6.80%	1.124±0.011 f* 4.55%

Values are expressed as Mean ± S.D. Lower case alphabet 'a' represents Chandigarh, 'b'=Gagret, 'c'= Daulatpur,'d'= Parwanoo, 'e' =Hamirpur, 'f'= Chintpurni and 'g'= Bangana. Mean ± S.D. is followed by Post hoc test of Bonferroni to compare the values of different regions with each other in which Means are statistically highly significant at $p<0.01$*, significant at $p<0.05$# and non significant above 0.05 =ns. It is followed by C.V. % (Coefficient of variation in percentage).

Table 5. Pearson correlation coefficients for 10 characteristics of dwarf honey bee (*Apis florea*) workers

	SpL	PdL	FgL	AtL	PoL	PmB	PmL	GL	LPL	TL
SpL	1	-0.003	-0.131	0.257	-0.061	0.026	0.101	0.18	0.07	-0.086
PdL	-0.003	1	-0.665	-0.365	-0.236	-0.181	-0.164	-0.234	-0.071	-0.112
FgL	-0.131	-0.665	1	0.619*	0.365	0.163	0.177	0.242	0.026	0.233
AtL	0.257	-0.365	0.619	1	0.159	0.044	0.111	0.14	-0.005	-0.2
PoL	-0.061	-0.236	0.365	0.159	1	0.307	0.211	0.226	0.042	0.189
PmB	0.026	-0.181	0.163	0.044	0.307	1	0.107	0.012	0.139	0.215
PmL	0.101	-0.164	0.177	0.111	0.211	0.107	1	0.283	-0.098	-0.212
GL	0.18	-0.234	0.242	0.14	0.226	0.012	0.283	1	0.093	0.106
LPL	0.07	-0.071	0.026	-0.005	0.042	0.139	-0.098	0.093	1	0.023
TL	-0.086	-0.112	0.233	-0.2	0.189	0.215	-0.212	0.106	0.023	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

observed differences between tongue length of *A. andreniformis* (2.797 mm) and *A. florea* (3.272 mm) from Thailand which helped to differentiate the two species. Niem and Trung (1999) reported proboscis length for *A. florea* from Vietnam (3.45 mm) and Thailand (3.57 mm) respectively. The PoL in tongue of *A. florea* from different populations was observed as Daulatpur (0.257 mm)>Gagret (0.242 mm) > Parwanoo (0.241 mm) > Chintpurni (0.237 mm) > Chandigarh (0.234 mm)

> Bangana (0.228 mm) respectively. The PmL in tongue of *A. florea* was lowest in bees from Chintpurni (1.027 mm) and showed significant difference (pd"0.01) with the bees of other populations. The PmB was lowest in bees from Chandigarh (0.409 mm) followed by Chintpurni (0.422 mm) < Bangana (0.427 mm) < Gagret (0.434 mm) < Daulatpur (0.439 mm) < Parwanoo (0.443) respectively. Niem and Trung (1999) reported 0.56 mm in width of prementum of *A. florea* from Vietnam and Thailand which was higher than that observed in the present case. Postmentum length was having weak positive correlation with width (r=0.307) of prementum.

Data for six characteristics namely length of pedicel, glossa, labial palp, postmentum and length and width of prementum of *Apis florea* is not reported till date.

In present study, 4 biometric indices have been studied to find out variations in the populations of *A. florea* from different regions (Table 5). The index was studied by Sharma (1983) who reported highest value for Kalka population which was different from the present findings. GL/PmL was highest in Gagret population and was significantly different from all other populations of *A. florea*. LpL/PmL exhibited highest value in Chintpurni (1.239) population and was significantly different from all other populations of *A. florea*. In similar studies Sharma (1983) reported highest value for Hamirpur population (1.440 mm) which was different from present studies.

These inter locality differences were significant ($p<0.01$) and could be related to altitudinal variations which significantly differed among seven regions and previously reported findings from the world. Our results also confirmed the findings of Tahmasebi et al. (2002) who reported that areas with higher altitudes have larger honey bees.

CONCLUSION

Data on tongue measurements revealed significant differences among samples of *Apis florea*. All the characteristics employed in the analysis proved to be of value in discriminating all the populations into two morphoclusters within the species of *A. florea* (Figure 4). Morphocluster A which was formed by the populations of Chintpurni and Bangana and morphocluster B formed by populations of Chandigarh, Gagret, Daulatpur, Parwanoo and Hamirpur *A. florea* population of morphocluster A belonged to regions located at highest altitude of the study area and were exposed to colder environmental condition with faster wind velocity and wild flora as forage source. Morphocluster B populations of *A. florea* belonged to lower altitude areas and had to face less cold climate, lower wind velocity and agricultural flora as forage source. Antennal characters exhibited significantly higher values in Daulatpur population of morphocluster B.

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Studies on the genus *Ambulyx* Westwood (Lepidoptera: Sphingidae) from India

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ABSTRACT: The male and female genitalic features of three species of genus *Ambulyx* Westwood viz. *substrigilis* (Westwood), *obliterata* Rothschild and *moorei* Moore have been studied and illustrated in detail. The genus diagnosis has been updated and a key to the species has been formulated.

KEYWORDS: A*mbulyx moorei, A. obliterata, A. substrigilis*, genitalia and sphingidae

INTRODUCTION

Rothschild and Jordan (1903) proposed the new genus *Oxyambulyx* for the placement of *substrigilis* Westwood and its allied species. Bell & Scott (1937) followed the same nomenclature. Earlier, Hampson (1892) discussed these species under genus *Ambulyx* Westwood. He even synonymized two other distinct genera i.e. *Dahira* Moore and *Clanis* Hübner under genus *Ambulyx*. Fletcher and Nye (1982) clarified the position and listed instances of the erroneous use of *Ambulyx* and synonymized *Oxyambulyx* as its junior synonym. Holloway (1987), Pittaway and Kitching (2000) followed the nomenclature recommended by Fletcher and Nye (1982). The same nomenclature has been adopted during the present studies. *Ambulyx* is most diverse in the Oriental Region from India to Sundaland but extends as far east as the Solomons (D'Abrera, 1986). Three species i.e. *substrigilis* (Westwood), *obliterata* Rothschild and *moorei* Moore have been studied in detail. The terminology for naming various genitalic features has been followed after Klots (1970). The critical examination of morphological characters including genitalic features revealed that these species conform to a natural group.

GENUS *AMBULYX* WESTWOOD

Westwood, 1847, *Cabinet Oriental Ent.*, 1847: 61; Hampson, 1892, *Moths India*, 1: 77; Fletcher & Nye, 1982, *The generic names of the moths of the world*, 4: 9.

Oxyambulyx, Rothschild & Jordan, 1903, *Novit. Zool.*, **9**: 192; Bell & Scott, 1937, *Fauna British India, Moths*, **5**: 109-113.

Type species: *substrigilis* (Westwood)

Distribution: World-wide.

Diagnosis: Labial palpus upturned, surpassing lower level of frons. Head with sharp inter-antennal crest. Proboscis reaching beyond end of abdomen. Antenna with end segments compressed, bottle

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shaped or conical in lateral view; variable in length, but at least four times as long as proceeding one; two bristles at end and several others in lateral and ventral surface; dorsal surface of segment covered with appressed scaling. Forewing with apex acute; dorsal one-fourth portion of anal vein forked; Cu_2 from middle of cell; Cu_1 from well before lower angle; M_3 from angle of cell; M_2 from below middle of discocellulars; M_1 from upper angle of cell or stalked as M_1 (R_5, R_4); $R_{(3+2)}$ from well before angle; discal cell less than half length of wing. Hind wing with both anals present; 2A forked at base; Cu_2 from beyond middle of cell; Cu_1 from well before lower angle; M_3 from lower angle of cell; M_1 and Rs from upper angle or stalked; discal cell one-third length of wing. Legs having mid tibia with one pair and hind tibia with two pairs of tibial spurs, inner ones longer; longer apical spur of hind tibia more than half length of first tarsal segment; tarsi spinose. Male genitalia with uncus undivided; apically bulbous, laterally compressed; saccular projection well developed, distal end well splayed; valva without friction scales. Female genitalia with basal half of ductus bursae usually guarded by well sclerotized genital plate.

Key to the species of genus *Ambulyx* Westwood:

1. Male genitalia with aedeagus with sclerotized wedge-shaped serrate plates; hind wing with veins M_1 and Rs from upper angle of cell, not stalked; abdomen with medial line on dorsal side..... *substrigilis* (Westwood) ..... Male genitalia with aedeagus without such plates or projections; hind wing with veins M_1 and Rs stalked from upper angle of cell; abdomen without dorsal line …….....…..2

2. Male genitalia with gnathos slightly notched at distal end; saccus reduced; forewing with a large antemedial spot; hind wing with veins Sc + R_1 anastomosing with cell beyond middle ..... *obliterata* Rothschild ..... Male genitalia with gnathos bifid; saccus oblong, well developed; forewing with four antemedial spots, costal one large; veins Sc + R_1 anastomosing with cell up to middle ..... *moorei* Moore

Ambulyx substrigilis (Westwood)

(Figs. 1-5)

Sphinx substrigilis Westwood, 1848, *Cab. Or. Ent.,* 1848: 61.

Ambulyx substrigilis, Walker, 1856, *List. Lep. Ins. B.M.,* 8: 122; Moore, 1865, *Proc. Zool. Soc. London,* 1856: 793; Butler, 1877, *Trans. Zool. Soc. London,* 9: 579; Hampson, 1892, *Moths India,* 1: 77. Fletcher & Nye, 1982, *The generic names of the moths of the world*, 3: 9. D'Abrera, 1986, *Sphingidae Mundi*, 1986: 55.

Oxyambulyx substrigilis, Rothschild and Jordan, 1903, *Novit. Zool.*, **9**: 202; Bell and Scott, 1937, *Fauna British India, Moths,* **5**: 131.

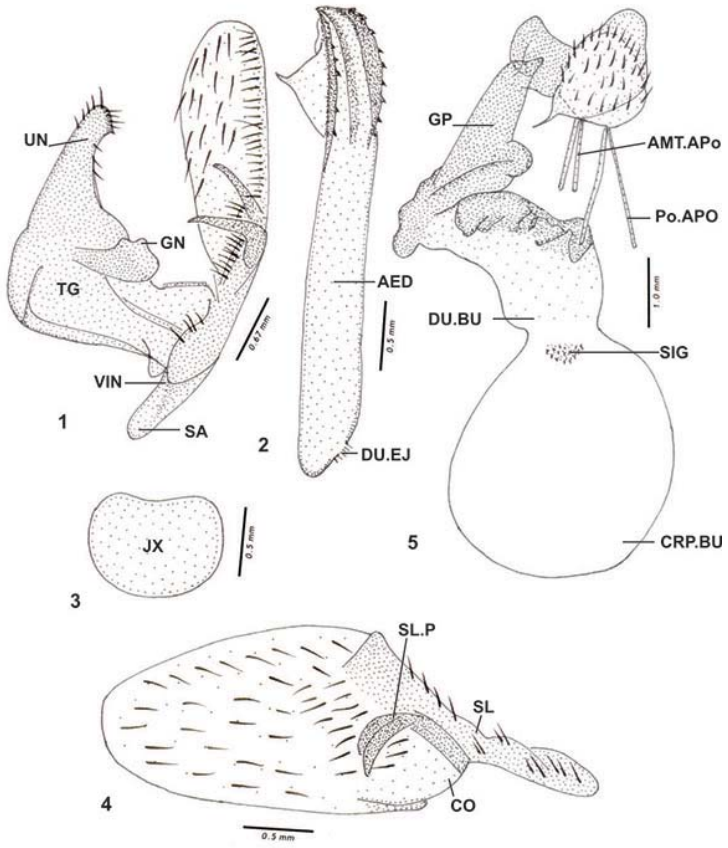
Wing Expanse: Male: 110 mm; Female: 120 mm.

Male genitalia**:** Uncus of moderate size, distal half laterally compressed, bulbous, tip highly sclerotized, beaked, setosed; gnathos reduced, semi-sclerotized; tegumen more than 2X length of vinculum, inverted U-shaped, slightly sclerotized; vinculum slightly sclerotized; saccus with rounded ending; juxta rounded, distal end emarginate in middle, slightly sclerotized; transtilla short, nearly membranous. Valva simple, extending well beyond level of uncus, costa not demarcated; sacculus moderately sclerotized, setosed; saccular projection broad, bifid, one arm broad, another one sickle-shaped, narrow, well sclerotized; distal half of valva broad, semi-membranous, setosed. Aedeagus long, well sclerotized; distal end having three wedge-shaped long, well sclerotized plates, two with serrate edges; vesica with semi-sclerotized small projection.

Female genitalia: Corpus bursae large, membranous; signum semi-lunulate; ductus bursae with anterior half broad, sclerotized, basal half narrow guarded by well sclerotized genital plate; anterior apophyses shorter than posterior ones, both pairs having rounded semi-membranous apices; papilla analis ovoid, fringed with short setae.

Material Examined: Arunachal Pradesh: West Kameng Distt., Bomdilla, 14.IX.1990, 1♂. Assam: North Cachar Hills, Jatinga, 03.IX.1991, 2♂♂;

PLATE - 1



***Ambulyx substrigilis* (Westwood)**
1. Male genitalia - lateral view; 2. Aedeagus; 3. Juxta - Ventral view;
4. Valva - Ventral view; 5. Female genitalia

05.IX.1991, 1♂; 06.IX.1991, 1♂. Karnataka: Bhagwati, 14.XI.2003, 1♂; Jog falls, 20.VII.1991, 1♂; 16.XI.2003, 1♂. Meghalaya: Jowaii, 14.IX.1991, 1♀.

Distribution: India - North West Himalayas, Arunachal Pradesh, Assam, Meghalaya, Sikkim; Else-where: Bangladesh, Bhutan, Borneo, Malaysia, Nepal, Philippines, Sri Lanka, Sumatra, Thailand and Vietnam.

Remarks: As discussed earlier, Fletcher and Nye (1982) revived the present species under its original genus. It is distinct from other two species examined in the present studies i.e. *obliterata* Rothschild and *moorei* Moore due to the origin of veins M_1 and Rs in hindwing and wedge shaped sclerotized projections in aedeagus. The collection of this species from two localities of Karnataka is its new distributional record from South India.

Ambulyx obliterata Rothschild

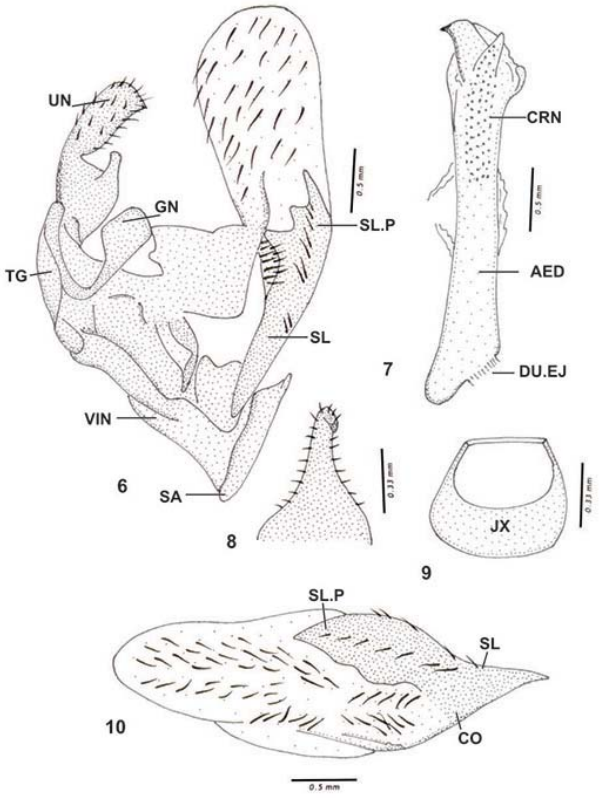
(Figs. 6-10)

Ambulyx liturata obliterata Rothschild, 1920, *Ann. Mag. nat. Hist,* **9**(5): 479.

Ambulyx obliterata Rothschild: Diehl, 1982, Heterocera Sumatrana, 1: 17, pl. 2: 18-19; Holloway, 1987, *Moths Borneo*, **3**: 129, pl.14: 11; Inoue *et al.*, 1997, Moths of Thailand, 2: 31-32.

Wing Expanse: Male: 100 mm; Female: Not examined.

PLATE - 2



***Ambulyx obliterata* Rothschild**
6. Male genitalia - lateral view; 7. Aedeagus; 8. Uncus - Ventral view;
9. Juxta - Ventral view; 10. Valva - Ventral view

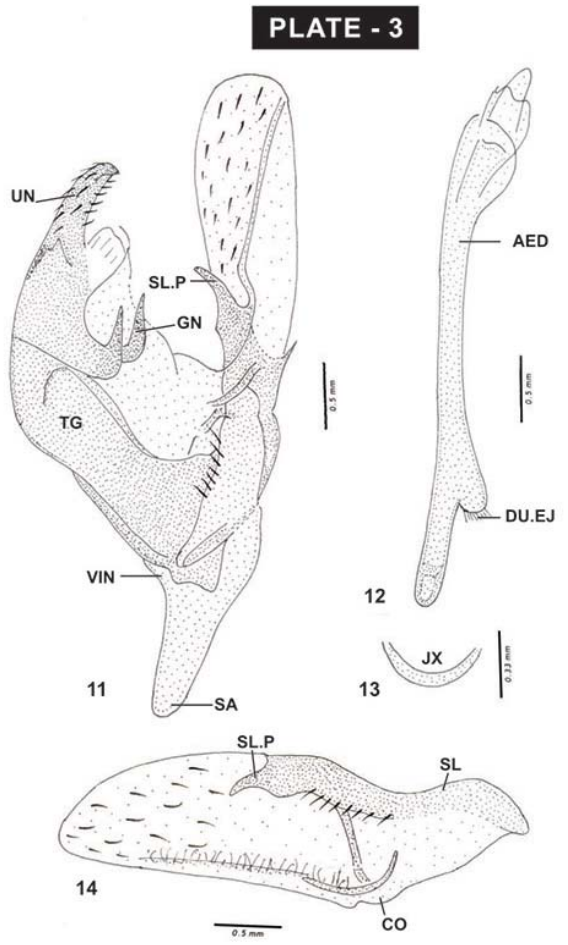
Male genitalia**:** Uncus having broad basal portion, distal portion laterally compressed, setosed with short setae, semi-sclerotized, beaked pointed tip; gnathos small, squarish, well sclerotized having slightly notched tip, extending up to level of base of uncus, subscaphium present; juxta with proximal end rounded, distal end strongly emarginate, semi-sclerotized; transtilla broad, triangular. Valva long, extending well beyond level of uncus; costa narrow, setosed, semi-sclerotized; sacculus well sclerotized having a well developed saccular projection, distal half with two lateral, outgrowths and one long projection having few long setae in the middle, distal portion of valva ovoid, semi-membranous, setosed. Aedeagus of moderate size, proximal end produced, distal end having a sclerotized plate with short pointed spur on one side; vesica armed with minute denticles.

Material Examined: Assam: North Cachar Hills, Jatinga, 03.IX.1991, 2♂♂; 06.IX.1991, 7♂♂.

Distribution: Borneo; Peninsular Malaysia; Sumatra.

Additional Distribution: India: Assam.

Remarks: It is characterized by large single antemedial spot in forewing and the wings are much lighter than in *liturata* Butler. Rothschild (1920) described it as a subspecies of *liturata* Butler, but Diehl (1982) upgraded its status as distinct species. Holloway (1987) and Inoue *et al.* (1997) followed him in this regard. It is characterized by large single antemedial spot in forewing and the wings are much lighter than in *liturata* Butler. Reporting of the present species from Assam is its first record from India.



***Ambulyx moorei* Moore**
11. Male genitalia - lateral view; 12. Aedeagus;
13. Juxta - Ventral view; 14. Valva - Ventral view

Ambulyx moorei Moore

(Figs. 11-14)

Ambulyx moorei Moore, [1858], in Horefield & Moore, *Cat. Lepid. Insects Mus. East-India Co.*, 1: 266; Kitching & Spitzer, 1995, *Tinea*, 14: 178; Inoue *et al.*, 1997, *Moths of Thailand*, 2: 35.

Ambulyx subocellata Felder, 1874, *Reise Ost. Fregatte Novara, Lep.*, 1874: 76; Holloway, 1987, *Moths Borneo*, 3: 82.

Oxyambulyx subocellata Felder, Rothschild and Jordan, 1903, *Novit. Zool.*, 9: 203; Bell and Scott, 1937, *Fauna British India, Moths*, 5: 136-138.

Wing Expanse: Male: 92-102 mm; Female: Not examined.

Male genitalia**:** Uncus well developed, broad at base, moderately sclerotized, setosed with small setae, curved, distal end highly sclerotized with beaked, pointed tip; gnathos simple, slightly sclerotized proximal half, distal half bifid, both arms narrow, well sclerotized with pointed tip; tegumen broad, inverted U-shaped, semi-sclerotized; vinculum narrow, shorter than tegumen, well sclerotized; saccus well developed, oblong; juxta narrow, semi-lunulate, semi-membranous; transtilla oblong, rounded semi-sclerotized. Valva quite long, extending well beyond level of uncus; costa narrow, semi-sclerotized; sacculus narrow, long, well sclerotized having a short saccular projection with swollen proximal half, narrow, falcate, short distal half, a backwardly pointing hook-like projection near costa, well sclerotized; distal portion of valva semi-membranous, setosed. Aedeagus narrow, proximal

end produced thumb-like, distal half broad, semi-sclerotized; vesica having rows of small denticles representing cornuti.

Material Examined: Arunachal Pradesh: West Kameng Distt., Bomdilla, 14.IX.1990, 1♂. Assam: North Cachar Hills, Jatinga, 03.IX.1991, 2♂♂; 06.IX.1991, 1♂; 07.IX.1991, 2♂♂. Karnataka: Bhagamandalam, 25.XI.2003, 1♂; Ganeshgudi, 13.XI.2003, 1♂; 14.XI.2003, 1♂; 15.XI.2003, 1♂. Himachal Pradesh: Sarahan, 17.VI.2000, 1♀.

Distribution: India - East Himalayas (Arunachal Pradesh, Assam), South India (Karnataka); Elsewhere: Borneo, Java, South China, Malaysia, Sumatra, Thailand and Vietnam.

Additional Distribution: Himachal Pradesh.

Remarks: Kitching and Spitzer (1995) synonymized the familiar name *subocellata* to *moorei* and described *Ambulyx moorei* Moore as a valid species. Inoue *et al.* (1997) followed the same nomenclature. The species under reference can be easily distinguished from other allied species due to the presence of four ocellate antemedial spots in forewing. It is being reported from North India for the first time.

ABBREVIATIONS:

AED: Aedeagus; ANT. APO: Anterior apophyses; CO: Costa; CRP. BU: Corpus bursae; DU. BU: Ductus bursae; DU. EJ: Ductus ejaculatoris; GN: Gnathos; JX: Juxta; PAP. A: Papilla analis; PO. APO: Posterior apophyses; SA: Saccus; SL: Sacculus; SL.P: Saccular projection; TG: Tegumen; UN: Uncus; VIN: Vinculum.

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Volatile organic compounds in healthy and *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) infested leaves of coconut palms

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ABSTRACT: An exploratory study, for the first time, was conducted to identify the volatile organic compounds (VOCs) present in dichloromethane extracts of uninfested and *O. arenosella* infested leaves of three varieties of coconut palms, viz. WCT, MGD and COD, as well as from the frass produced by the caterpillars while feeding on these varieties. Many VOCs reported from other plant species and having specific functions in plant-plant and plant-insect interactions were identified in the leaf material. Green leaf volatiles (Z)-3-hexen-1-ol and (Z)-3-hexen-1-ol acetate, acetophenone and nonanal were found in both uninfested and infested leaves. Differences in VOCs were observed between different varieties of coconut plants and between infested and uninfested plants of these varieties. The VOCs identified in insect frass included 6-hydroxy-2-hexanone, n-hexadecanoic acid, β-pinene, β-myrcene, hexahydrofarnesyl acetone, acetophenone and undecane. Indicated possible roles of the identified VOCs based on existing reports for other plant species.

KEYWORDS: GC-MS analysis, green leaf volatiles, herbivore-induced plant volatiles

INTRODUCTION

The coconut palm, *Cocos nucifera* L., is regarded in tropical countries as the 'Tree of Life' (Foale, 2003). The coconut cultivation industry in India directly or indirectly employs approximately 12 million people and contributes US $1.28 billion to the GDP of the country (Thomas, 2013). Coconut production is threatened by more than 800 species of pests in India and Sri Lanka (Singh and Rethinam, 2006). *Opisina arenosella* Walker (Lepidoptera: Oecophoridae), the black-headed caterpillar pest of coconut, is a serious defoliator of coconut palms in the entire coastal belt of Kerala and many parts of the states of Tamil Nadu and Karnataka. In severe outbreaks, thousands of palms can be affected with as much as 90% of leaf damage (Cock and Perera, 1987; Mohan and Sathiamma, 2007) and crop losses up to 45.4% in terms of nut yield from infested palms in the succeeding year of

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severe pest incidence (Chandrika *et al*., 2010). Current control methods include chemical control by using pesticides such as monocrotophos or dicrotophos (Rao *et al*., 1981) and biological control by releasing larval (*Goniozus nephantidis* Muesebeck), prepupal (*Elasmus nephantidis* Rohwer) and pupal parasitoids (*Brachymeria nosatoi* Habu) (Sathiamma *et al.*, 1987). However, an alternative and environmentally friendly approach is to exploit the plants' own defence mechanisms against herbivores.

The leaves of all plants normally release small quantities of volatile organic compounds (VOCs) known as constitutive volatiles. However, upon herbivore attack, the quantity and composition of volatile blends will change leading to the emission of herbivore-induced plant volatiles (HIPVs) (Pare and Tumlinson, 1999; Arimura *et al.,* 2009). Common VOCs emitted by plants include the 'green-leaf volatiles' (C6 aldehydes, alcohols, and derivatives), cyclic and acyclic terpenes, phenolics (aromatics), and sulphur- or nitrogen-containing compounds (Dicke, 1999; Dudareva *et al.,* 2004; Arimura *et al.,* 2009). These volatiles play multiple roles in plant defense, including herbivore repellence and deterrence, attraction of natural enemies, allelopathy and protection against abiotic stressors such as radiation and harmful gases (Holopainen, 2004; Kant *et al.*, 2009; Hare, 2011).

There is a great variability in the composition of volatile blends between plant species, and even among different plant genotypes and varieties within the same plant species (Gouinguene *et al*., 2001; Degen *et al.*, 2004). Volatile emission is dynamic, changing through the course of the herbivory, and can vary depending on the herbivore species attacking, its abundance and developmental stage (De Moraes *et al.*, 1998; Girling *et al.*, 2011; McCormick *et al*., 2014). Both herbivores and their natural enemies make use of the information conveyed by volatile blends to locate and infer the quality of their host plant or prey (Bruce *et al*., 2005; Dicke, 2000; McCormick *et al*., 2012; Mumm and Dicke, 2010).

The use of VOCs, especially the HIPVs, has gained importance in modern agricultural pest management practices (Yu *et al*., 2008; Orre *et al.*, 2010). For example, in sweet corn, HIPVs attracted many parasitoids belonging to Eulophidae and Encyrtidae, which significantly reduced the sweet corn pest *Helicoverpa* spp. infestation (Simpson *et al*., 2011). Information on the VOCs released by healthy and infested coconut palms and coconut pests is scarce and only few studies have been done (Bakthavatsalam *et al*., 1999; Shameer *et al*., 2002; Subaharan, 2008). Hence, an exploratory study was conducted to identify the volatile organic compounds (VOCs) present in healthy/ uninfested and *O. arenosella* infested leaves of three varieties of coconut palms, viz. WCT, MGD and COD, and the frass of larval galleries on the leaves of these varieties of coconut palms. Many VOCs have been identified and reported for the first time on these varieties of coconut palms. We reviewed the literature to discuss possible roles of these VOCs.

MATERIALS AND METHODS

Coconut palms

Three varieties of coconut palms, viz. West Coast Tall (WCT), Malayan Green Dwarf (MGD) and Chowghat Orange Dwarf (COD) were used for the present study. Two to three-years-old healthy coconut seedlings of WCT, MGD and COD varieties bearing 3-4 leaves were procured from the State Agriculture Department Coconut Nursery at Anakkayam, Kerala. The seedlings were transferred into clay pots (35 x 35 cm) with rich nutrient soil. Dried and powdered cow dung (bio-fertilizer) (250 gm) and a fertilizer mixture (100 gm of N, P and K in the proportion of 40-40-20) (Manufactured by MCP Agro Technologies (P) Ltd., Kerala and procured from the Fertilizer Depot of FACT, Kerala) were added to each seedling at 6-month intervals. The seedlings were watered daily and kept in an insect-proof net house.

Rearing *O. arenosella* in the laboratory

The larvae of all instars of *O. arenosella* collected from infested palms in the field were transferred

into 500 ml glass beakers (Borosil, India) with freshly cut coconut leaves and covered with cotton cloth fastened with rubber bands. The larvae were transferred into new beakers every forty-eight hours with freshly cut coconut leaves till pupation and the pupae were kept in 250 ml glass beakers (Borosil, India) until the adults emerged. The emerged moths were allowed to mate for one day and released into caged leaves of coconut seedlings to maintain the insect rearing.

Since *O. arenosella* moths prefer to lay eggs in the larval galleries made of silken threads and frass, infested leaves with galleries collected from the field were cut into pieces (10-15 cm long) and 6-8 pieces of old galleries were pinned on the under surface of the undetached coconut leaf on the seedling. The leaf was enclosed within the net cage with the open end of the cage tied to the petiole of the leaf with jute twine and mated *O. arenosella* moths were introduced into the net cage. After 30 days of releasing the moths, the leaf was cut at the base of the petiole and larvae were carefully transferred into 500 ml glass beakers with fresh pieces of leaves. The larvae were transferred every two days to sterilized beakers with fresh pieces of leaves and seventh instar larvae were used for the experiments.

Extraction of volatile organic compounds from leaves of coconut palms

Collection of volatiles from healthy leaves

Fresh leaflets from 3-4 years-old coconut seedlings were cut at the base, washed in running water and cleaned with absorbent cotton to remove dust particles, if any. The leaflets were soaked in HPLC-Grade Dichloromethane (99.5%, Merck, India) for 12 hours (10 g in 50 ml DCM) (Scascighini *et al.*, 2005). The solvent extract was concentrated to 2 ml in Rotor Evaporator and filtered and decolorized by passing through Glasswool – anhydrous Sodium Sulphate (Na_2SO_4) - activated Charcoal column. The clear extracts were taken in Agilent glass tubes (2 ml) with Teflon caps (Agilent Technologies, U.S.A.) and stored at –60° C till analysis of the samples. The extraction was done simultaneously from the leaves of five individual plants of each of the three varieties of coconut.

Collection of volatiles from *O. arenosella* infested leaves

Twenty-five seventh instar *O. arenosella* larvae were introduced into a caged fresh leaf. The larvae were allowed to feed for 24 hours. After removing the frass and larvae carefully with a paintbrush, the volatiles were extracted from the infested leaves of three varieties of coconut palms separately using the same procedure as followed in the case of healthy leaves (Scascighini *et al.*, 2005).

Collection of volatiles from Larval Frass

The larval frass removed from the fed leaves of three varieties of coconut palms was separately soaked in DCM in the same proportion as done in the case of uninfested and infested leaves.

The extracts collected were labeled as follows -

WUL – WCT Uninfested Leaf; WIL – WCT Infested Leaf; WLF – WCT Larval Frass (Larval frass of *O. arenosella* on WCT); MUL – MGD Uninfested Leaf; MIL – MGD Infested Leaf; MLF – MGD Larval Frass (Larval frass of *O. arenosella* on MGD); CUL – COD Uninfested Leaf; CIL – COD Infested Leaf; CLF – COD Larval Frass (Larval frass of *O. arenosella* on COD).

Identification of volatiles in Gas Chromatography coupled Mass Spectrometry (GC-MS)

Five replicates of each variety of uninfested and infested leaves and frass of larvae were run in the GC-MS, and we only report compounds present in all replicates. One µl of the concentrated extract was injected in splitless mode into Agilent Gas Chromatograph (Model GC6890 N coupled with a HP 5975 B Mass Selective Detector). HP 5 Column was used with helium as a carrier gas.

During the run the temperature programme was as follows: 40°C for 3 min and rise at 5° C / min to 280° C, isotherm for 10 min; Post run 10 min at 300° C. The injector and column temperatures were 250° C. The total run was for 23 min. The retention time and mass spectra were compared with MP and NIST libraries. For compound identification, we report the best match with the NIST library, however, compound identity needs to be further confirmed against authentic standards.

RESULTS

A number of compounds belonging to various groups like green leaf volatiles, hydrocarbons, alcohols, aldehydes, acids, esters, ketones, terpenes, aromatic compounds etc. were identified in each variety of coconut palms. All the compounds appeared at retention times between 4.212 min. and 19.21 min.

Uninfested leaves of WCT, MGD and COD varieties

The uninfested leaves of all the three varieties of coconut palms produced a C6 compound, which is also a green leaf volatile, viz. (Z)-3-Hexen-1-ol. Whereas, other green leaf volatiles viz. (Z)-3-Hexen-1-ol acetate was present in MUL and CUL, and 2,4-dimethyl-Hexane was present in MUL. Heptacosane, a long chain hydrocarbon appeared in the compounds present in WUL and CUL. Apart from these, 2-Hexen-4-olide, Phenyl ethyl Alcohol, Isopropyl Myristate and Hexahydrofarnesyl acetone were obtained from WUL. β-Myrcene, a natural organic hydrocarbon classified as monoterpene, was present in MUL. Nonanal (a C9 aldehyde), Acetophenone and 1-Docosene were present in CUL (Table 1).

Table 1. Volatile organic compounds obtained in GCMS analysis of uninfested leaves of different varieties of coconut

Variety of coconut	RT (min.)	Compound	Class of compound
WUL	5.279	(Z)-3-Hexen-1-ol	Green leaf volatile
	6.324	2-Hexen-4-olide	Other
	7.92	Phenylethyl Alcohol	Alcohol
	13.128	Isopropyl Myristate	Ester
	13.309	Hexahydrofarnesyl acetone	Ketone
	16.249	Heptacosane	Long chain hydrocarbon
MUL	5.279	(Z)-3-Hexen-1-ol	Green leaf volatile
	6.696	β-Myrcene	Monoterpene
	6.792	(Z)-3-Hexen-1-ol acetate	Green leaf volatile
	7.056	o-Cymene	Aromatic
	7.678	2,4-dimethyl- Hexane	Other
CUL	5.274	(Z)-3-Hexen-1-ol	Green leaf volatile
	6.791	(Z)-3-Hexen-1-ol acetate	Green leaf volatile
	7.497	Acetophenone	Aromatic
	7.755	Nonanal	Aledhyde
	16.267	Heptacosane	Long chain hydrocarbon
	17.938	1-Docosene	Long chain hydrocarbon

RT - Retention Time, WUL – WCT Uninfested Leaf, MUL – MGD uninfested Leaf, CUL – COD Uninfested Leaf

Opisina arenosella infested leaves of WCT, MGD and COD varieties

(Z)-3-Hexen-1-ol and Heptacosane were obtained from the infested leaves of WIL and MIL, whereas Methyl Benzoate was present in WIL. (Z)-3-Hexen-1-ol acetate, Heneicosane, Nonanal and Decanal were isolated from MIL. 2,6-Dimethoxy benzoquinone was obtained from both MIL and CIL and aromatic compounds like p-Cymene and Acetophenone were obtained from CIL (Table 2).

Larval frass of *O. arenosella* reared on leaves of WCT, MGD and COD varieties

Monoterpenes like β-Pinene were found in WLF and CLF, whereas β-Myrcene was obtained from MLF. Aromatic compounds like p-Cymene was obtained from WLF and CLF and m-Cymene, o-Cymene and Acetophenone, were present in MLF and CLF. Compounds which were earlier reported as pheromone components of various other insects were also obtained from the frass of *O. arenosella*

Table 2. Volatile organic compounds obtained in GCMS analysis of *O. arenosella* infested leaves of different varieties of coconut

Variety of coconut	RT (min.)	Compound	Class of compound
WIL	4.657	Cyclobutene, 2-propenylidene	Other
	5.269	(Z)-3-Hexen-1-ol	Green leaf volatile
	7.745	Methyl benzoate	Aromatic
	7.855	Cyclohexane, 2-ethenyl-1, 1-dimethyl-3-methylene	Other
	15.527	Pterin-6-carboxylic acid	Carboxyilic acid
	16.25	Heptacosane	Long chain hydrocarbon
MIL	5.269	(Z)-3-Hexen-1-ol	Green leaf volatile
	6.789	(Z)-3-Hexen-1-ol, acetate	Green leaf volatile
	7.752	Nonanal	Aledhyde
	8.667	Decanal	Long chain aldehyde
	11.609	2,6-Dimethoxy benzoquinone	Other
	13.3	Hexahydrofarnesyl acetone	Ketone
	13.76	Methyl palmitate	Ester
	18.055	Heneicosane	Long chain hydrocarbon
	19.21	Heptacosane	Long chain hydrocarbon
CIL	6.786	p-Cymene	Aromatic
	7.489	Acetophenone	Aromatic
	7.749	1-Heptanol, 2-propyl-	Other
	11.765	2,6-Dimethoxy benzoquinone	Other
	14.035	n-Hexadecanoic acid (palmitic acid)	Fatty acid
	15.266	Oleic acid	Fatty acid

RT - Retention Time, WIL – WCT Infested Leaf, MIL – MGD Infested Leaf, CIL – COD Infested Leaf

Table 3. Volatile organic compounds obtained in GCMS analysis of larval frass of *O. arenosella* reared on different varieties of coconut

Variety of coconut	RT (min.)	Compound	Class of compound
WLF	4.212	3-methyl-3-Hexanol	Other
	4.368	Cyclobutene, 2-propenylidene	Other
	6.664	β-Pinene	Monoterpene
	7.049	p-Cymene	Aromatic
	13.306	Hexa -hydrofarnesyl acetone	Ketone
	13.554	Hexa-hydro-farnesol	Alcohol
MLF	5.645	6-hydroxy-2-Hexanone	Other
	6.707	β-Myrcene	Monoterpene
	7.052	m-Cymene	Aromatic
	7.49	Acetophenone	Aromatic
	13.309	Hexahydrofarnesyl acetone	Ketone
CLF	6.673	β-Pinene	Monoterpene
	6.751	p-Cymene	Aromatic
	7.05	o-Cymene	Aromatic
	7.49	Acetophenone	Aromatic
	7.675	Undecane	Long chain hydrocarbon
	8.314	Methyl nicotinate	N containing compound
	14.079	n-Hexadecanoic acid (palmitic acid)	Fatty acid
	15.267	Oleic acid	Fatty acid
	15.419	Phytol	Diterpene alcohol

RT - Retention Time, WLF – Larval Frass on WCT, MLF – Larval Frass on MGD, CLF – Larval Frass on COD

reared on different varieties of coconut plams. Hexa -hydrofarnesyl acetone and Hexa-hydro-farnesol, were obtained from WLF; 6-hydroxy-2-Hexanone, was obtained from MLF and the long chain hydrocarbon, Undecane, was obtained from CLF (Table 3).

DISCUSSION

The present study revealed the presence of many potential VOCs in healthy and *O. arenosella* infested leaves of three varieties of coconut palms and frass of the larvae of *O. arenosella.* Though compound identification was based on the best match to the NIST library, compounds could be accurately classified to chemical group level (e.g. green leaf volatile or terpenoid) by the characteristics of their mass spectra. Since many VOCs in WCT, MGD and COD varieties of coconut palms are reported for the first time in the present study, e.g. β-Myrcene in MUL and Nonanol in CUL, previous references are lacking to check whether these compounds are released by all the varieties.

Some compounds, like (*Z*)-3-hexenol are present in uninfested leaves of all varieties, whereas others such as (*Z*)-3-hexenyl acetate are found in two of the three varieties (MUL and CUL). Other compounds appear to be unique to a specific variety, for instance β-myrcene in MUL or nonanol in CUL, indicating that there are varietal differences in the

odour profiles (Table 1). The herbivore-induced profiles show even more differences among varieties (Table 2). Similar differences on healthy and infested leaf volatile profiles have been reported for other plant species like cotton (Loughrin *et al.,* 1995) and barley (Pettersson *et al.*, 1999). The volatiles emitted from the vegetative parts of plants, especially those released after herbivory, may protect plants by deterring herbivores and by attracting the natural enemies of herbivores (Pichersky and Gershenzon, 2002).

(Z)-3-Hexen-1-ol, (Z)-3-Hexen-1-ol acetate and 2,4-dimethyl- Hexane, which were obtained from the leaves of coconut palms are Green Leaf Volatiles (GLVs), C_6 aldehydes, esters and carbons released after mechanical wounding (Arimura *et al.*, 2009). These GLVs, produced or emitted upon herbivory by almost every green plant, are used by insects as semiochemicals and are reported to be involved in indirect and direct plant defenses of many plant species (Scala *et al*., 2013). Subaharan (2008) reported that (Z)-3-hexen-1-ol was present in both damaged and undamaged leaves of coconut palms. Matsui *et al.* (2012) have reported that (Z)-3-hexenol and (Z)-3-hexenyl acetate are produced in the intact parts of partially wounded leaves. The green-leaf volatile, (Z)-3-hexenyl acetate, and other aliphatic esters of (Z)-3-hexen-1-ol that are emitted by tobacco after damage were found to deter female *Heliothis virescens* moths from laying eggs on injured plants (Moraes *et al*., 2001). Several examples of (Z)-3-hexenyl acetate-mediated plant communication have also been reported (Prost *et al.*, 2005; Engelberth *et al.*, 2004; Heil and Kost, 2006).

Terpenes are also an important group of plant volatiles serving multiple roles in plant defense such as antimicrobial activities, protection against abiotic stress and plant-insect communication (Cheng *et al*., 2007; Gershenzon and Dudareva, 2007; Pichersky *et al*., 2006). The uninfested leaves of MGD variety produced β-Myrcene, which is a monoterpene and a common constitutive volatile of many plants having possible antimicrobial activity (Laouer *et al.,* 2009). The frass of *O. arenosella* larvae also contained monoterpenes like β-Pinene and β-Myrcene. Both are common constitutive volatiles produced by plants and possibly involved in antimicrobial activity, which possibly remain in the larval frass after the ingestion of plant material (Dorman and Dean, 2000; Laouer *et al.,* 2009). Subaharan (2008) reported the presence of α-pinene in damaged leaves and larval frass, and β-pinene in undamaged and damaged leaves, but the variety of coconut palm used for the study is unknown. Terpenoids can also play a role in indirect defense by attracting natural enemies of herbivores, for example feeding by *Pieris rapae* larvae induces *Arabidopsis thaliana* to emit a blend of volatiles consisting of terpenoids that is attractive to a larval parasitoid of *P. rapae* (Van Poecke *et al*., 2001).

Aromatic compounds have simple aromatic rings and C1–C3 side, one of the most important representatives of the group is methyl salicylate which plays important roles in plant defense and signaling (Loake and Grant, 2007; Park *et al*., 2007). Members of this group such as Acetophenone, Methyl Benzoate and p-Cymene appeared in the infested leaf samples. Acetophenone is reported to be a possible insect attractant in the flowers of red clover plant *(Trifolium pratense* L.) (Buttery *et al.,* 1984), whereas Methyl Benzoate is a common floral volatile involved in the pollinator attraction, e.g. in common garden snapdragon, *Antirrhinum majus* (Pischerski and Gershenzon, 2002) and p-Cymene was reported to have a possible role in plant-insect interactions, e.g. it acts as a repellent in tomato (*Solanum lycopersicum*) against the whitefly, *Bemisia tabaci* (Bleeker *et al.,* 2009). Aromatic compounds like Acetophenone, p-Cymene, m-Cymene and o-Cymene were also obtained from the frass of *O. arenosella*. m-Cymene and o-Cymene have been reported as having antioxidant and antimicrobial properties in *Citrus acida* Roxb. against bacteria such as *Bacillus subtilis* and fungi such as *Candida utilis* (Mahmud *et al.,* 2009).

Insect-derived hydrocarbons have major ecological roles mediating species- and gender- recognition and nest mate recognition; and are used as task-specific cues, dominance and fertility cues, and primer pheromones (Howard and Blomquist, 2005).

For instance, Heptacosane has been reported to be a sex pheromone component of several insect species (El-Sayed, 2016), and Heneicosane, was also reported as a pheromone for attracting the female *Aedes aegypti* (Bhutia *et al*., 2010). Long chain hydrocarbons can also be emitted by plants and mediate plant-insect interactions. For instance, Heneicosane, obtained from healthy and infested leaves during the present study has been previously reported in maize volatile extracts which mediate plant-insect interactions (Krokos *et al*., 2002), whereas another long chain hydrocarbon, 1-Docosene, identified in healthy leaves is reported to be a floral volatile and possible insect attractant in *Clusia* (Guttiferae) plants (Nogueira *et al*., 2001).

Long chain aldehydes like nonanal appeared in both un-infested and infested leaves and decanal appeared in the infested leaf only. Nonanal has been frequently reported as an important component of volatile blends involved in insect attraction (Cha *et al*., 2008; Fraser *et al*., 2003; Metcalf and Kogan, 1987). Other examples are: nonanal, released from whitefly infected beans (*Phaseolus vulgaris*) (Birkett *et al*., 2003), was also reported to induce resistance against bacterial pathogen, *Pseudomonas syringae* in Lima bean (*Phaseolus lunatus*) plants (Yi *et al*., 2009). Nonanal and Decanal released from apple trees elicited high EAG response in *Cydia pomonella* (Gonzalez, 2007).

Compounds belonging to other chemical classes were also found in the frass and leaf material. For instance, the infested leaves of MGD and COD varieties produced 2,6-dimethoxy benzoquinone, which is reported to be involved in plant-plant communication, e.g. it induces haustorium development in parasitic plants in the *Scrophulariaceae* to invade the roots of neighboring plants (Yoder, 2001) and can also have antibiotic activities ((Nishina *et al*., 1991), e.g. 2,6-dimethoxy-1,4-benzoquinone enhances resistance against rice blast fungus *Magnaporthe oryzae* (Ueno and Yoshikiyo, 2014). Frass samples contained Hexa-hydro-farnesyl acetone and Hexa-hydro-farnesol which have been reported as pheromone components reported from many Hymenopteran insect species, such as *Geotrigona mombuca*, *Apis dorsata* (both Apidae) (El-Sayed, 2016). Hexa-hydro-farnesyl acetone was also reported as an attractant in male orchid bee, *Euglossa* spp. (Eltz *et al*., 2010) and also reported to induce antimicrobial activity in the Algerian *Phlomis bovei* De Noé against pathogens (Liolios *et al*., 2007).

It is evident from the present study that the leaves of coconut palms produced many VOCs, which have been reported to have important functions in other plants and insects. Many of the VOCs produced by uninfested and infested coconut palms and frass are herbivore-induced plant volatiles (HIPVs), that can mediate both direct and indirect defenses acting as herbivore deterrents, or attracting the foraging carnivorous predators and parasitoids to kill the herbivores (De Moraes *et al*., 2001; Dicke *et al*., 2009; Mumm and Dicke, 2010; McCormick *et al*., 2012).

Ghosh and Abdurahiman, as early as 1996, suggested that the kairomones emitted from the larval gallery of *O. arenosella* might serve as an important factor in host searching and oviposition behaviour of early larval parasitoid *Apanteles taragamae* Viereck (Hymenoptera: Braconidae). Subaharan (2008) reported that the damaged coconut leaflet odors elicited more antennal response in the late larval parasitoid *Goniozus nephantidis* Muesebeck (Hymenoptera: Bethylidae) than the undamaged leaflets, however the identity of the compounds and blends responsible for this response remains unknown.

The role of VOCs produced by coconut palms in either direct or indirect defence has not been investigated so far and hence, we cannot attribute any functions to particular VOCs identified from the coconut leaves. However, earlier reports on other plants and insects suggest that the VOCs identified from coconut leaves might have important roles in coconut palm – *O. arenosella* – interactions with herbivores and their natural enemies. Further studies are required to investigate the differences between the volatiles profiles of infested and uninfested plants, and behavioral assays with

herbivores and their natural enemies are required to establish the role of the identified compounds in the interactions between the coconut palms and the insect community.

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A new species of *Psilocera* Walker (Hymenoptera: Pteromalidae) from Eastern Ghats, Tamil Nadu, India with a key to the Oriental species

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ABSTRACT: A new species of Pteromalidae, *Psilocera manickai* sp.nov.has been described from Eastern Ghats, Yercaud, Tamil Nadu, India. Key to the Oriental species of *Psilocera* is provided.

KEY WORDS: Pteromalidae, *Psilocera*, new species, Key to Oriental species, Eastern Ghats, India

INTRODUCTION

The genus *Psiloera* Walker belongs to the subfamily Pteromalinae of family Pteromalidae which is currently known by 31 described species world wide with ten species from the Oriental region (Noyes, 2017, Sureshan, 2014). The genus *Psilocera* contains two species groups, one with a normal scutellum and the other with the scutellum produced in the form of hump with a distinct finger nail-like tip. Among the Oriental species, *P. clavicornis* (Ashmead), *P. intermedia* Sureshan, *P. neocalvicornis* Narendran and Girish Kumar, *P. heydoni* Sureshan and *P. scutellata* Sureshan bear humped scutellum and *P. keralensis* Sureshan, *P. ghanii* Subba Rao, *P. vinayaki* Sureshan and Narendran, *P. clavata* Sureshan and Narendran and *P. namdaphaensis* Sureshan bear a normal scutellum. Major contributions towards the taxonomy of Oriental *Psilocera* are Rao (1981), Sureshan (2000, 2001, 2014), Sureshan and Narendran (1995) and Narendran and Kumar (2009). In this paper a new species of *Psilocera* with a normal scutellum is described based on the specimens collected from Yercaud which is located in the Shevaroy hills, Eastern Ghats of Tamil Nadu. The affinities of the new species with closely related species are discussed and a modified key to the Oriental species of *Psilocera* is provided.

MATERIALS AND METHODS

The specimens of the present study were collected in yellow pan trap from Yercaud located in the Shevaroy hills of Salem district, Tamil Nadu. They were preserved in 70% ethyl alcohol and card mounted for microscopic observation. They were studied and macrophotographed under Leica M 205 C sterezoom trinocular microscope mounted with LeicaMC 170 HD camera. The specimens are deposited in the National Zoological Collections of Zoological Survey of India, Kozhikode.

The following abbreviations are used in the text: F1-F6- Funicular segments 1-6; MV- marginal vein; OOL- ocellocular distance; PMV- postmarginal

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vein; POL- postocellar distance; SMV- submarginal vein; STV- stigmal vein, T1-T5– tergites1-5 of gaster; ZSIK- Zoological Survey of India, Western Ghat Regional Centre, Kozhikode. Terminology of morphology follows Boucek (1988) except the terms mesosoma and metasoma are used for thorax and gaster, respectively.

RESUTLS

Psilocera Walker

Psilocera Walker, 1833: *Ent.Mag*.1: 373. Type species: *P. obscura* Walker by monotypy.
Acanthometopon Ashmead, 1904: *Mem.Car. Mus*.1 (4):314,315,498. Type species
A. clavicorne Ashmead, by monotypy and original designation. Syn. by Sureshan, 2001:35:84.
For further synonymy see Graham,1969.

Diagnosis: Head distinctly wider than mesosoma, moderately reticulate except a narrow elevated area between clypeus and toruli faintly reticulate to smooth. Vertex narrow, occiput abruptly sloping, not carinate. Clypeus with two triangular teeth. Antennae inserted below middle of face, in female flagellum strongly clavate with 2 or 3 anelli, clava with large area of micropilosity, sutures oblique. Male flagellum very long, filiform, with 6-8 pedunculate segments bearing whorls of strong setae. Mesosoma strongly arched, pronotum narrower than mesoscutum, anteriorly carinate mainly in the middle. Mesoscutum with notauli incomplete. Scutellum highly convex, sometimes with a conical hump bearing a finger nail-like tip, length of the finger nail varies. Propodeum finely reticulate, strongly constricted into a nucha, median carina and costula distinct. Gaster short to moderate, acuminate, petiole smooth, hardly longer than broad, hind margin of basal tergites incised in the middle, petiole in male sometimes a little longer than wide.

Key to the Oriental species of *Psilocera* Walker (Females)

(Modified from Sureshan, 2014)

1. Scutellum with a conical hump bearing a finger nail like tip ……..……..…..……..**2**

- Scutellum normal without conical hump ……..**6**

2. Scutellum with long hump and finger nail sharp and strongly projecting…….....…..…..…**3**

- Scutellum with short hump, finger nail not much sharp and projecting as above…….....…**5**

3. Forewing with basal cell hairy and closed below (Brazil, Sri Lanka) ……. …….....***P. clavicornis*** **(Ashmead)**

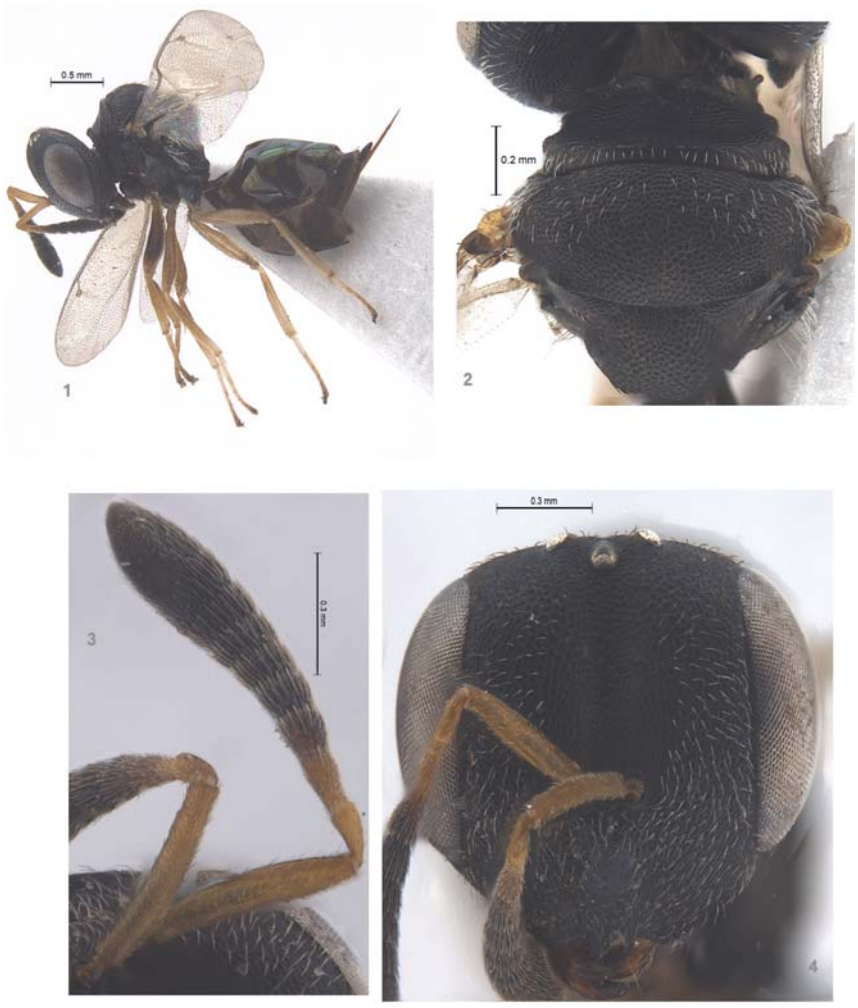
- Forewing with basal cell not hairy as above, sometimes a few hairs at tip, and open below…….....…...**4**

4. Antenna with pedicel as long as F1; clava shorter than 4 preceding segments combined; scape, pedicel, anelli and basal three-fourths of F1 testaceous; gaster 1.72× as long as hind tibia and 0.9× rest of the body. (India)..…. ***P. neoclavicornis*** **Narendran & Girish Kumar**

- Pedicel almost 2× F1; clava longer than four preceding segments combined; F1 black as remainder of antenna; gaster 1.4× as long as hind tibia and 0.7× as long as rest of the body (India)……...***P. intermedia*** **Sureshan**

5. Scutellar hump short, median length of scutellum up to tip of hump 0.73× length of mesoscutum; hind tibial spur long, almost half as long as basitarsus; forewing with PMV 0.72× as long as MV; eye short, height 1.6× width; gaster dorsally with metallic blue reflection; antenna with scape, pedicel and anelli brown; legs with femora brown (India)….. ***P. heydoni*** **Sureshan**

- Scutellar hump long, median length of scutellum up to tip of hump 0.82× length of mesoscutum, hind tibial spur short, 0.3× as long as basitarsus; forewing with PMV 0.9× MV; eyes longer, height 1.9× width; gaster dorsally without blue reflection; antenna with scape, pedicel and anelli testaceous; femora testaceous (India) ….....***P. scutellata*** **Sureshan**



Figs. 1-4. *Psilocera manickai* sp. nov. 1. Female - body in profile view; 2. Mesosoma in dorsal view; 3. Antenna; 4. Head in front view

6. Antenna with F1 short or anelliform, 1.2-1.4× as broad as long, and without sensilla; scape reaching far below level of median ocellus ……..……...**7**

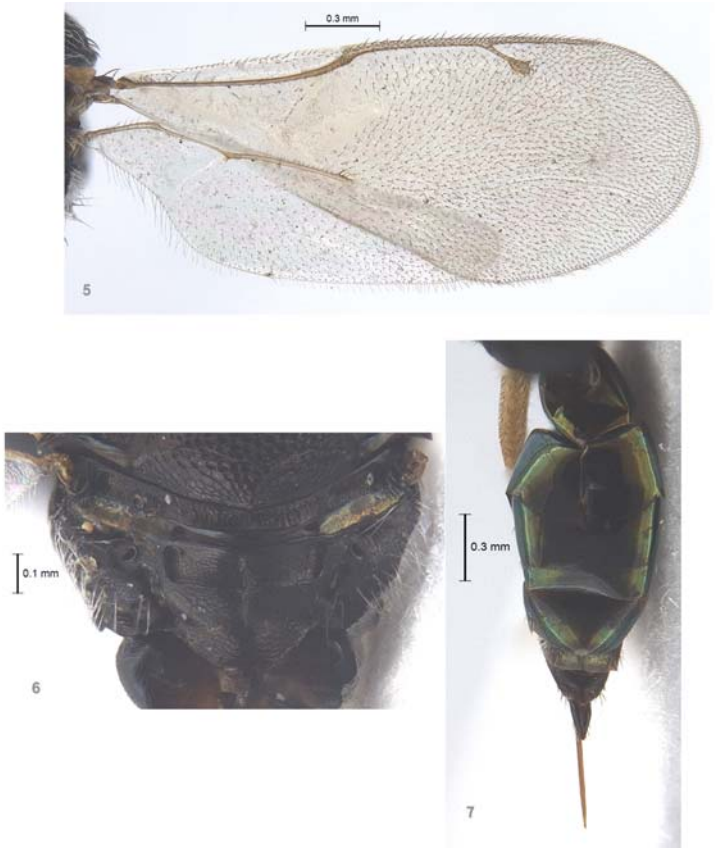
- Antenna with F1 not short or anelliform, longer than broad, and with distinct sensilla; scape just short of reaching or just touching median ocellus…..…….....……**8**

7. Antenna with F1 very short, anelliform, 0.3× as long as F2 and 1.4× as broad as long, flagellum with clava little swollen; antenna black except scape, pedicel and anelli testaceous (India)… ……..... ***P. keralensis*** **Sureshan**

- Antenna with F1 not very short as above, 1.2× as broad as long and only little shorter than F2, clava highly swollen, antenna testaceous except clava black (India) …….. **.....*P. clavata* Sureshan & Narendran**

8. Malar groove not distinct; antenna with clava shorter than three preceding segments combined; scutellum without long hairs or bristles (Pakistan).....…..…....***P. ghanii*** **Subba Rao**

Malar grooves distinct; clava as long as or longer than three preceding segments combined; scutellum with long white hairs or black bristles ……..……..……**9**



Figs. 5-7. ***Psilocera manickai*** **sp. nov. 5. Forewing; 6. Propodeum in dorsal view; 7. Gaster in dorsal view**

9. Clava as long as three preceding segments combined; scape reaching median ocellus; scutellum with long white bristles (India)..... .....***P. vinayaki*** **Sureshan & Narendran**

- Clava as long as 3.5 preceding segments combined; scape just short of reaching median ocellus; scutellum with long black bristles ……..**10**

10. Pedicel 1.9× as long as broad, T2 0.5× as long as gaster medially, gaster 0.7× as long as head plus mesosoma combined; propodeum 2.6× as broad as long in dorsal view (India)…….... ..... ***P. namdaphaensis*** **Sureshan**

- Pedicel 2.5× as long as broad, T2 0.4× as long as gaster medially, gaster as long as head plus mesosoma combined; propodeum 3× as broad as long and medially 0.6× as long as scutellum (India)… ……...….***P.manickai*** **sp.nov.**

Psilocera manickai **sp.nov.**

LSID urn:lsid:zoobank.org:act:B6EF555F-C235-4CCA-98D3-F856CA546425

(Figs.1-7)

Holotype Female: Length 3.73mm (Paratype-3.52mm). Head and mesosoma black without metallic reflections, gaster brownish black with slight bluish reflections dorsally and brown on ventral part; antenna with scape, pedicel, anelli and F1 testaceous, remainder brownish black; eyes grey; ocelli silvery; mandibles blackish brown; tegula brown; wings hyaline, slightly smoky, veins and pubescence brown; coxae concolorous with mesosoma, fore femora brown, rest of legs testaceous with tips of tarsi brown.

Head: (Figs.1,4) in dorsal view 2.2× as broad as long, POL 1.13× OOL, temple length 0.5× eye length, vertex sharply declivitous, moderately reticulate, vertex and genal area closely reticulate, a broad shiny area above clypeus, pubescence

white, dense on lower half of face. Head in front view width 1.3× height; eyes separated by 1.3× their height at the level of toruli; malar space 0.51× eye height in front view; clypeus radiately striated, striae not reaching much beyond outer margin, anterior margin with two sharp teeth; scrobe deep reaching median ocellus; scape (Fig. 3) length 0.7× eye length, pedicel as long as F1, anelli transverse, second slightly thicker than first , pedicel plus flagellum length 0.83× head width, flagellum moderately clavate, F1 as long as F2, , F1-F3 longer than broad, F4-F6 transverse, all funicular segments with one row of long sensillae, clava 2.2× as long as broad, as long as 3.5× preceding segments combined, sutures oblique, micropilosity area reaching up to base of third segment.

Mesosoma: (Figs.1,2) Highly convex, pubescence and brown bristles sparse; pronotal collar narrow, finely and transversely reticulate, anterior margin finely and sharply carinate in the middle. Mesoscutum and scutellum distinctly punctuate reticulate, mesoscutum width 2.2× length, notauli incomplete; scutellum as long as mesoscutum without conical hump; frenal area slightly raised in the central point, frenum clearly separated; axilla and axillula finely reticulate; dorsellum narrow, very finely and transversely reticulate. Propodeum (Fig.6) 0.6× as long as scutellum, in dorsal view 3× as broad as long, finely reticulate, median carina and costuladistinct and complete, post spiracular sulcus deep, callus with long dense hairs; prepectus as long as tegula, almost shiny; mesopleuron distinctly reticulate with a broad triangular shiny area below hind wings; metapleuron moderately reticulate. Legs slender, hind coxa 1.5× as long as broad, femora 5× as long as broad, tibia almost as long as femora, hind tibia with two spurs. Forewing (Fig.5) 2.33× as long as broad, marginal fringe very small, pubescence moderate, basal cell open below, basal hairline indicated, speculum open below. Relative length SMV-1, MV- 0.64, PMV-0.42, STV-0.185.

Metasoma: (Fig.7) Petiole shiny and distinct, ventrally supported by the extension of first sternite. Gaster lanceolate, dorsally collapsing, as long as head plus mesosoma combined, hind margin of T1-T3 deeply incised, T4 elongate.

Male: unknown

Material Examined: Holotype: Female, India: Tamil Nadu, Yercaud (11.7794°N &78.2034°E, elevation 1515m), 6.iii.2014, Coll.Manickavasagam, Reg.No. ZSI/WGRC/IR/INV/8093. Paratype: one female, data same as that of holotype. Reg.No. ZSI/WGRC/IR/INV/8094.

Host: Unknown.

Etymology: The species is named in honor of Dr.Manickavasagam, Professor, Department of Entomology, Faculty of Agriculture, Annamalai University for his valuable contributions to the studies of Indian Chalcidoidea.

Remarks:In the key to the Oriental species of *Psilocera* Walker by Sureshan (2014) this species runs to couplet 9 and closely resembles *P. namdaphaensis* Sureshan in general morphology but differs from it on the basis of following characters (characters in brackets are those of *namdaphaensis*) : Antenna with pedicel 2.5× (1.9×) as long as broad, gaster as long as head plus mesosoma combined (0.7×), T1 medially 0.4× (0.5×) as long as gaster, propodeum 3× (2.6×) as long as broad in dorsal view and medially 0.6× (0.8×) as long as scutellum and size ranging from 3.52 to 3.73 mm (3 mm). This species also resembles *P. vinayaki* Sureshan & Narendran in in general morphology but differs from it on the basis of following characters (characters in brackets are those of *vinayaki*): Antenna with F1 length 1.58× its width (1.8×), claval width 0.5× length (0.4×), propodeum 0.6× as long as scutellum (0.8×), gaster 1.48× as long as mesosoma (0.96×), clypeus radiately striated, striae not reaching much beyond outer margin (striae extending laterally up to little beneath the eyes), frenum clearly separated (frenum vaguely indicated).

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Survey and documentation of Pyraloidea fauna associated with horticulture crops of zone-1 and 2 of Karnataka, India

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ABSTRACT: Pyraloidea is the third largest super family of the order Lepidoptera, and has great economic importance as it causes serious damage to crop plants as a borers, root feeders, seed feeders, leaf rollers and webbers. Survey and documentation of Pyraloidea fauna from their actual hosts is the need of hour for accurate identification and authentication of its host. Survey and documentation of Pyraloidea fauna occurring on horticulture crops of zone-1 and 2 of Karnataka, India revealed a total of 22 identified and 5 unidentified species of Pyraloidea from 711 specimens collected and reared on their respective hosts falling under 20 genera, representing 5 sub-families *viz*., Phycitinae, Epipaschiinae, Spilomelinae, Glaphyriinae and Cybalomiinae. Among 5 sub-families, two sub-families Epipaschiinae and Phycitinae were belonging to Pyralidae, while remaining three sub-families were belong to Crambidae.

KEY WORDS: Pyraloidea, horticulture crops, Karnataka, survey, documentation

INTRODUCTION

Karnataka is divided into 10 agro-climatic zones by considering the rainfall pattern, soil types, topography and major crops grown *etc*. The zone-1 (Eastern-transition zone) and zone-2 (North-Eastern dry zone) comprises of 4 districts namely, Bidar, Kalaburagi, Yadagir and Raichur with two districts under each zone, respectively. The major horticultural crops growing in these zones include mango, banana, sapota, brinjal, chilli, onion, cucurbits, zinger, turmeric *etc*., with an area of 0.064 M. ha which represents 3.36 per cent of total horticultural area of Karnataka (Anon., 2014). Among various biotic stresses, the damage and yield loss caused by insect pests are main contributory factor. Insect pests of Pyraloidea have great economic importance as many of them cause serious damage either internally as borers, root feeders and seed feeders or externally as leaf rollers or webbers (Munroe and Solis, 1999; Solis, 1997 and Solis, 2007). The extent of yield loss due to Pyraloidea ranged from 10 to100 per cent across the world (Usua, 1968; Jotwani and Young, 1972).

Most of the pyralid taxonomists have undertaken faunistic studies predominantly by relying on light trap collections and they did not made any efforts

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to associate Pyraloidea species with their host plants except Nagaraj (2014) who made a first effort to survey and document the Pyraloidea fauna associated with major cereals of Hyderabad-Karnataka region. The description of species reared from actual hosts is the need of the hour for accurate identification and authentication of its host. In the zone-1 and 2 of Karnataka, the information pertaining to the fauna of Pyraloidea associated with horticultural crops is not available. In this context, an attempt has been made to survey and documentation of Pyraloidea fauna associated with horticulture crops from zone-1 and 2 of Karnataka, India.

MATERIALS AND METHODS

Intensive collections of Pyraloidea occurring on horticultural crops were made by undertaking survey in different localities of zone-1 (Bidar, Humnabad, Kalaburagi, and Raddewadgi) and zone-2 (Naganoor, Kavadimatti, Raichur and Chandrabanda) of Karnataka once in month from August 2015 to January 2016. The collected specimens transferred to rearing plastic containers / wooden cages along with its host, was monitored / examined carefully twice a day and fresh food was provided to the larvae until attaining pupal stage. Later, pupae were collected and kept for adult emergence in wooden cages / plastic boxes. The rearing room was disinfected with two per cent formaldehyde at regular interval to maintain the hygiene. The emerged adults were killed immediately by using ethyl acetate, pinned, stretched, dried, labeled properly and preserved in insect cabinet boxes at insect repository, Department of Entomology, Agriculture College, Bheemarayanagudi, India. The collected specimens were identified to generic and species level based on the keys developed by Hampson in the Moths volumes of the Fauna of India and adjacent countries series and also using recently available literature (Hampson, 1896).

RESULTS AND DISCUSSION

During the survey, a total of 22 identified and 5 unidentified species of Pyraloidea were documented, out of 711 specimens collected and reared on their respective hosts (Table 1). All the identified and unidentified species were belongs to 20 genera, representing 5 sub-families *viz*., Phycitinae, Epipaschiinae, Spilomelinae, Glaphyriinae and Cybalomiinae. The sub-family Epipaschiinae documented with an identified species, *Orthaga exvinacea* Walker and an unidentified species under genus *Lepidogma* Meyrick. While the sub-family Phycitinae was documented with three species namely, *Etiella zinckenella* Treitschke, *Euzophera perticella* Ragonot, *Nephopterix eugraphella* Ragonot and an unidentified species under genus *Nephopterix* Hübner. Likewise, the sub-family Spilomelinae recorded with 14 species *viz*., *Palpita vitrealis* Rossi, *Syllepte lunalis* Gunee, *Maruca vitrata* Fabricius, *Cirrhochrista brizoalis* Walker, *Diaphania indica* Saunders, *Glyphodes vertumnalis* Guenée, *Omiodes indicata* Fabricius, *Leucinodes orbonalis* Guenée, *Spoladea recurvalis* Fabricius, *Spoladea perspectalis* Hübner, *Conogethes punctiferalis* Guenée, *Synclera univocalis* Walker, *Nausinoe geometralis* Guenée and *Nausinoe perspectata.* The sub-family also comprises three unidentified species under three genera namely, *Conogethes* Meyrick, *Synclera* Lederer and *Nausinoe* Hübner. The sub-family Glaphyriinae was documented with three species namely, *Noorda blitealis* Walker, *Noorda moringae* Tams and *Crocidolomia pavonana* Fabricius. While, the sub-family Cybalomiinae was documented with single species *Hendecasis duplifascialis* Hampson. Similarly, Bhattacharjee (1962) made extensive surveys on Indian Pyralidae for his *Ph.D.* research work, who collected 35 species grouping to 20 genera. In another study, Rose (1982) collected 93 species of pyralid moths falling under 61 genera of sub-family Pyraustinae from North India. Likewise, recently Nagaraj (2014) surveyed for Pyraloidea associated with cereals from Hyderabad-Karnataka region who documented 7 identified and 6 unidentified species. Similar results were also reported by various authors (Rose, 2001; Kirti and Sodhi, 2001; Landry and Brown, 2005; Li, 2006; Landry, 2008; Guillermet, 2008; Du, 2008; Mey, 2008; Qi *et al.* 2011; Sharma, 2011; Jiayu and Houhun, 2012; Li,

Table 1. Species of Pyraloidea collected through survey and reared on horticultural crops from zone-1 and 2 of Karnataka

Insect species	Sub family	Host plant	No.	Remarks
Orthaga exvinacea Walker	Epipaschiinae	Mango	63	Leaf webber
Nephopterix eugraphella Ragonot	Phycitinae	Sapota	65	Leaf webber/ fruit borer
Nephopterix sp.	Phycitinae	Sapota	2	Leaf webber/ fruit borer
Synclera univocalis Walker	Spilomelinae	Ber	2	Leaf webber
Synclera sp.	Spilomelinae	Ber	1	Leaf webber
Syllepte lunalis Gunee	Spilomelinae	Grapevine	41	Leaf webber
Lepidogma sp.	Epipaschiinae	Jamun	29	Leaf webber
Cirrhochrista brizoalis Walker	Spilomelinae	Fig	4	Fruit borer
Spoladea recurvalis Fabricius	Spilomelinae	Amaranthus	28	Leaf webber
Spoladea perspectalis Hübner	Spilomelinae	Amaranthus	1	Leaf webber
Leucinodes orbonalis Guenée	Spilomelinae	Brinjal	111	Shoot and fruit borer
Euzophera perticella Ragonot	Phycitinae	Brinjal	49	Stem borer
Diaphania indica Saunders	Spilomelinae	Cucurbits	59	Leaf webber
Crocidolomia pavonana Fabricius	Glaphyriinae	Cabbage	2	Leaf webber
Omiodes indicata Fabricius	Spilomelinae	Field bean	37	Leaf webber
Maruca vitrata Fabricius	Spilomelinae	Field bean	12	Flower webber
Etiella zinckenella Treitschke	Phycitiinae	Field bean	2	Pod borer
Noorda blitealis Walker	Glaphyriinae	Moringa	24	Leaf webber
Noorda moringae Tams	Glaphyriinae	Moringa	40	Bud borer
Nausinoe geometralis Guenée	Spilomelinae	Jasmine	66	Leaf webber
Nausinoe perspectata Fabricius	Spilomelinae	Jasmine	3	Leaf webber
Nausinoe sp.	Spilomelinae	Jasmine	1	Leaf webber
Palpita vitrealis Rossi	Spilomelinae	Jasmine	21	Leaf webber
Hendecasis duplifascialis Hampson	Cybalomiinae	Jasmine	8	Bud borer
Glyphodes vertumnalis Guenée	Spilomelinae	Jasmine	6	Leaf webber
Conogethes punctiferalis Guenee	Spilomelinae	Guava	18	Fruit borer
Conogethes punctiferalis Guenee	Spilomelinae	Mango	3	Inflorescence borer
Conogethes punctiferalis Guenee	Spilomelinae	Pomegranate	3	Fruit borer
Conogethes sp.	Spilomelinae	Guava	1	Fruit borer
Conogethes sp.	Spilomelinae	Pomegranate	3	Fruit borer
Conogethes sp.	Spilomelinae	Amaranthus	6	Inflorescence borer
Total			**711**	

2012; Sumpich and Skyva, 2012; Yonglin and Houhun, 2012; and Zhang, 2012) across the world.

The documentation of species reared from their actual hosts is the need of the hour for accurate identification and authentication of its host. So, in the current study, Pyraloidea associated with horticultural crops were studied and documented. On jasmine, five species of Pyraloidea were recorded *viz*., *Nausinoe geometralis* Guenée, *Nausinoe perspectata* Fabricius, *Palpita vitrealis* Rossi, *Glyphodes vertumnalis* Guenée and

Hendecasis duplifascialis Hampson. And also, an unidentified species under genus *Nausinoe* Hübner was documented. On leafy vegetable like amaranths, *Conogethes punctiferalis* Guenée, *Spoladea recurvalis* Fabricius, *Spoladea perspectalis* Hübner and an unidentified species under genus *Conogethes* Meyrick were recorded. While on brinjal, field beans and moringa, two species under each were documented namely, *Leucinodes orbonalis* Guenée and *Euzophera perticella* Ragonot, *Maruca vitrata* Fabricius and *Omiodes indicata* Fabricius, *Noorda blitealis* Walker and *Noorda moringae* Tams, respectively. On fruit crops like ber and guava, recorded with single species namely *Synclera univocalis* Walker and *Conogethes punctiferalis* Guenée, respectively. And an unidentified species was also documented under each. Likewise, on other fruit crops like grapes and fig, and vegetables like cabbage were recorded with single species under each *viz*., *Syllepte lunalis* Gunee, *Cirrhochrista brizoalis* Walker and *Crocidolomia pavonana* Fabricius, respectively. While on jamun, one unidentified species was documented.

The current study was the first of its kind that we attempted to survey and document the Pyraloidea taxa purely based on their hosts from zone-1 and 2 of Karnataka. Thus, this host based taxonomy of Pyraloidea helps in authentication of its host. During the survey, a total of 22 identified and 5 unidentified species of Pyraloidea were recorded out of 711 specimens collected and reared on their respective hosts. All the identified and unidentified species belong to 20 genera, representing 5 sub-families *viz*., Phycitinae, Epipaschiinae, Spilomelinae, Glaphyriinae and Cybalomiinae.

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Genitalia study on the genus *Glyphodes* (Crambidae: Spilomelinae) in Tamil Nadu, India

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ABSTRACT: Survey carried out to collect species belonging to the genus *Glyphodes* in different places of Tamil Nadu, *viz.*, Coimbatore, Anaikatti, Ooty, Yercaud, Kodaikanal and Periyakulam recorded a total of six species of the genus *Glyphodes viz., G. bivitralis, G. caesalis, G. canthusalis, G. onychinalis, G. pulverulentalis and G. stolalis.* Male and female genitalia of the collected species are described.

KEYWORDS: Distribution, *Glyphodes*, Spilomelinae, genitalia, Tamil Nadu

INTRODUCTION

Spilomelinae is the largest subfamily in the Crambidae, with about 3,767 species worldwide (Regier *et al*., 2012) and are of economic importance as many species cause serious damage to agricultural and horticultural crops, forests trees and ornamental plants (Mathew and Menon, 1984). Under Spilomelinae the genus *Glyphodes* Guenée consists of 120 species and is widespread in tropical regions, with some species penetrating into subtropical and warm temperate areas (Common, 1990; Robinson *et al.*, 1994). Twenty five species have been recorded in the Southeast Asia and 17 species in Australia (Robinson *et al.*, 1994; Shaffer *et al.*, 1996). In Tamil Nadu,three species were recorded by Fletcher (1914) and Nair (1970) are *Glyphodes bivitralis*, *G. caesalis*, *G. canthusalis*. Before this background, the present study aims to identify the *Glyphodes* species encountered in different regions of Tamil Nadu by studying morphological characters of the genitalia, and to record the distribution of those species.

MATERIALS AND METHODS

The study was conducted in Coimbatore, Anaikatti, Ooty, Yercaud, Kodaikanal and Periyakulam areas of Tamil Nadu, India during 2014-15. Moths were attracted using a white cloth (1.5 x 5.5 m) and a mercury lamp (400 Watts) from 6.00pm to 6.00am.The moths were killed with ethyl acetate and transferred into butter paper covers. The insects were curated and labeled as per Johnson and Triplehorn (2005). The moths were identified as per Hampson (1896, 1898) and with the reference collection of the Insect Biosystematics Laboratory, TNAU, Coimbatore. The generic and species nomenclature followed as per Beccaloni *et al*. (2003) and Nuss *et al*. (2003-2015).Taxonomically informative characters *viz*., antennae, labial palpi, forewing (FW), hindwing (HW), legs, tympanum, male and female genitalia were studied. The standard technique given by Robinson (1976) was followed for genitalia studies, while wing venation was studied using the Comstock – Needham system (1898). Genitalia images were taken in a

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Leica MZ16 stereozoom microscope. Illustrations were drawn by using mirror type camera lucida Leica M80.

RESULTS AND DISCUSSION

During the study, six species of the genus *Glyphodes* were collected from light trap: *Glyphodes bivitralis* (Yercaud, Coimbatore), *G. caesalis* (Anaikatti, Yercaud), *G. canthusalis* (Coimbatore), *G. onychinalis* (Coimbatore, Yercaud, Kodaikanal),*G. stolalis* (Coimbatore, Yercaud, Kodaikanal), *G. pulverulentalis* (Coimbatore, Killikulam, Kodaikanal, Periyakulam); the later was also recorded from its host plant (mulberry).

Common characters of the six investigated species: Filiform antennae; labial palpi upturned; maxillary palpi and basally scaled proboscis present. In the forewing, vein R_3 stalked with R_4; R_5 free; 1A+2A complete with a basal fork and 3A short, forming a loop. In hind wing, $Sc+R_1$ thickened, basally anastomosed, with Rs after its origin from upper angle of cell and ends at costa near apex. The male and female genitalia of six species are described below.

Glyphodes bivitralis Guenée, 1854

Male genitalia: Uncus long, anteriorly enlarged with constricted tip; uncus tip with five short spines, ventrally fringed with long hairs. Gnathos absent; subscaphium long, strap-like.Tegumen long, sclerotized, dome-shaped and arched; vinculum short; saccus broadly U-shaped.Valva long, membranous; apex broadly fringed with hairs; costa inconspicuous; medially sclerotized line with distinct thickening; sacculus broad at base, ridge-like, dorsally bent inward; harpe long, claw-like basally supported with medial sclerotized line; hair pencil with bunch of short hairs. Transtilla composed of triangular anterior projections meeting at mid line; juxta narrow, flap-like. Phallus long, sclerotized thread-like (Fig.1a).

Female genitalia: Anal papillae densely setose. Anterior apophyses long, basally with angular projection; posterior apophyses short when compared to anterior apophyses.Ostium oval, membranous; antrum short, moderately sclerotized; ductus seminalis originate at posterior end of ductus bursae above antrum. Ductus bursae very long, narrow and membranous; corpus bursae oval, membranous; signum composed of two denticulate, sclerotized signa (Fig. 2a)

Glyphodes caesalis Walker, 1859

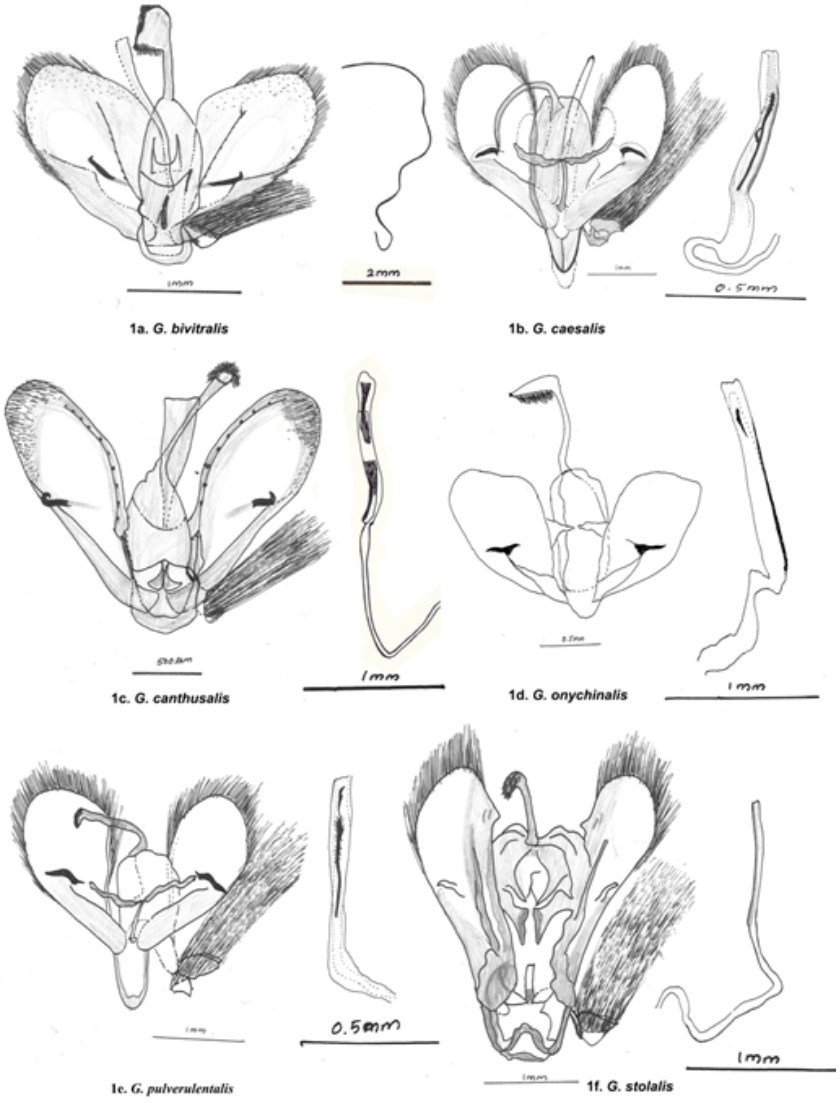
Male genitalia: Uncus long and narrow, apex enlarged and pointed, tip fringed with hairs. Gnathos absent; subscaphium long, strap-like.Tegumen longer than wide, sclerotized and arched; vinculum long, sclerotized and saccus long, U shaped. Valva long, membranous, apex narrow and rounded; costa inconspicuous, dorsally fringed with long hairs; sacculus broad, weakly sclerotized; ridge like dorsal edge with fine setae; harpe long, spine-like, sclerotized. Transtilla long, sclerotized band-like meeting at mid line; juxta narrow, sclerotized, arrow-like. Phallus almost straight, vesica with long sclerotized bar with lateral spine-like projection; medially long, curved sclerotized hook-like cornuti (Fig.1b).

Female genitalia: Anal papillae fringed with both long and short setae. Anterior apophyses long, basally with angular projection; posterior apophyses short.Ostium oval, membranous. Antrum short, sclerotized; ductus seminalis originate at posterior end of ductus bursae below antrum. Ductus bursae long and wide, membranous; caudally constricted; corpus bursae oval, membranous; sclerotized dots forming two strip-like signa (Fig.2b).

Glyphodes canthusalis Walker, 1859

Male genitalia: Uncus long, narrow, anterior tip enlarged and round, dorsally fringed with long setae. Gnathos absent, subscaphium long, strap-like, uniformly sclerotized.Tegumen long, membranous; vinculum long, curved and saccus broadly U shaped. Valva long, medially widened and apex narrow fringed with hairs; costa sclerotized, prominent with series of spots; sacculus broad at base, narrowed anteriorly and prominent; harpe long, sclerotized,

Plate 1. Male genitalia of the genus *Glyphodes*



1a. *G. bivitralis* 1b. *G. caesalis*

1c. *G. canthusalis* 1d. *G. onychinalis*

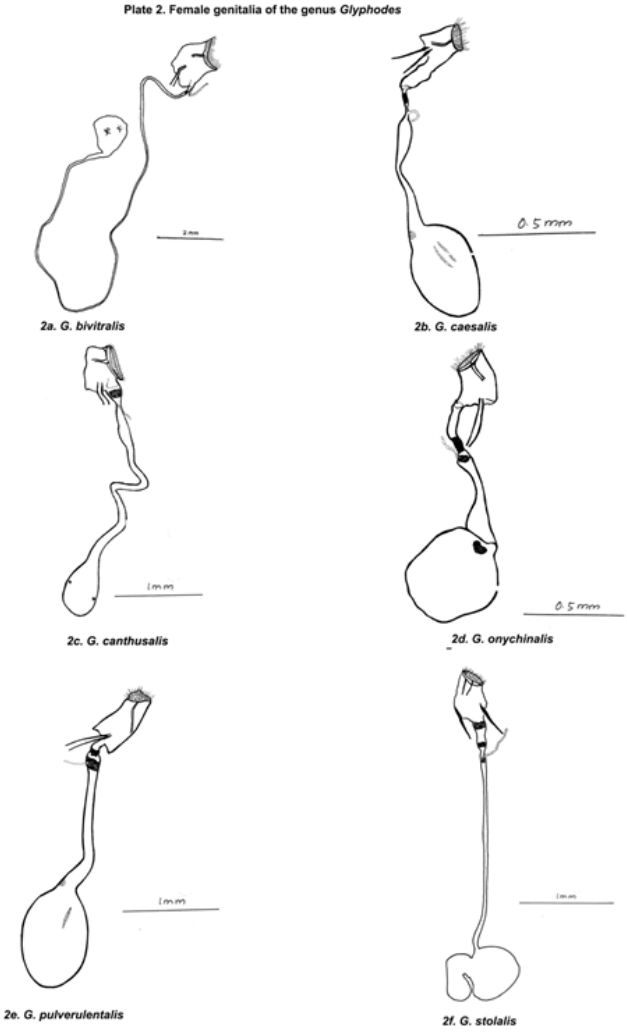
1e. *G. pulverulentalis* 1f. *G. stolalis*

claw-like; upturned apically. Transtilla membranous; juxta broad with anterior weakly sclerotized projection. Phallus long, vesica with intermittent patch-like sclerotization (Fig. 1c).

Female genitalia: Anal papillae densely fringed with setae. Anterior apophyses longer than posterior apophyses with medial angular projection. Ostium funnel shaped, membranous; ductus seminalis originate at posterior end of ductus bursae. Ductus bursae long, membranous; corpus bursae elongate, membranous; two small patches of sclerotized spines forming signa (Fig. 2c).

***Glyphodes onychinalis* (Guenée, 1854)**

Male genitalia: Uncus long, anterior half strongly curved, enlarged and beak shaped, ventrally fringed with long hairs; anterior tip with four short spines. Gnathos absent; subscaphium long, strap-like.Tegumen longer than wide, arched; vinculum almost long, weakly sclerotized and saccus broadly U shaped. Valva long, apex narrow; outer margin covered with black hairs; costa inconspicuous; sacculus narrow, ridge-like, weakly sclerotized; harpe long, spine-like; directed distally and supported basally by the apical tip of the



sacculus. Transtilla composed of lateral projections meeting mid line; juxta narrow, flap-like and weakly sclerotized. Phallus almost straight, strongly sclerotized on ventral side and vesica with sclerotized patch-like cornutus (Fig.1d).

Female genitalia: Anal papillae densely setose. Anterior apophyses long, basally with angular projection; posterior apophyses short.Ostium oval, membranous.Antrum broadly sclerotized; ductus seminalis originate at antrum. Ductus bursae long, wide at anterior and membranous; corpus bursae round, membranous with heart shaped signum (Fig. 2d).

***Glyphodes pulverulentalis* Hampson, 1896**

Male genitalia: Uncus long and narrow, anterior tip enlarged and pointed, beak-shaped dorsally with short setae. Gnathos absent; subscaphium long, strap-like.Tegumen longer than wide, sclerotized and arched; vinculum long, sclerotized and saccus long, U shaped. Valva long, membranous, apex broadly rounded; costa weakly sclerotized, dorsally fringed with long hairs; sacculus broad, weakly sclerotized; ridge-like, dorsal edge with fine setae; harpe long, spine-like; sclerotized, directed distally. Transtilla long, sclerotized band-like meeting at mid line; juxta narrow, sclerotized, arrow-like. Hair pencil with long bunch of hairs; phallus almost straight, vesica with long sclerotized bar with lateral spine-like projection; apically with long, curved sclerotized hook-like cornutus (Fig.1e).

Female genitalia: Anal papillae oval, fringed with both long and short setae. Anterior apophyses long, basally with angular projection; posterior apophyses short. Ostium membranous; antrum broadly sclerotized; ductus seminalis originate from antrum. Ductus bursae long, membranous; corpus bursae oval, membranous; signum as two strip-like sclerotized dots (Fig. 2e).

***Glyphodes stolalis* Guenée, 1854**

Male genitalia: Uncus long and narrow, anterior tip enlarged and spoon shaped, fringed with setae. Gnathos absent; tegumen longer than wide, arched and teguminal ridges strongly sclerotized; vinculum almost long, sclerotized and saccus broadly W shaped. Valva long, narrow at base with broad apex, outer margin fringed with long hairs; costa inconspicuous, sclerotized, distally with triangular dorsal projection; sacculus narrow, ridge-like; harpe long, sclerotized and spine-like. Transtilla sclerotized flap-like extending downward; juxta short, membranous.Hair pencil with short bunch of hairs. Phallus straight, long; phallus apodeme strongly sclerotized on ventral side; cornuti absent (Fig. 1f).

Female genitalia: Anal papillae oval, fringed with short and long setae. Anterior apophyses long,

Plate 3. Identified species of the genus *Glyphodes*

3a. ***G. bivitralis***

3b. ***G. caesalis***

3c. ***G. canthusalis***

3d. ***G. onychinalis***

3e. ***G. pulverulentalis***

3f. ***G. stolalis***

basally broad; posterior apophyses short. Ostium oval, weakly sclerotized; antrum weakly sclerotized; ductus seminalis originate at posterior end of ductus bursae below antrum. Ductus bursae long, narrow; corpus bursae rounded, membranous; accessory bursae present, dropper shaped with minute denticulation; signum absent (Fig. 2f).

Identification key to investigated species of *Glyphodes*

1. FW and HW with spots and striations; ♀ genitalia with signum, appendix bursae absent …….. 2

1a. FW and HW with striations; ♀ genitalia without signum, appendix bursae present ……. ***Glyphodes stolalis***

2. ♀ genitalia with denticulate or stripe-like signum ……..... 3

2a. Heart shaped signum..... ***Glyphodes onychinalis***

3. Uncus long and narrow; tip enlarged ……. 4

3a. Uncus beak shaped; tip with five short spines ……..... ***Glyphodes bivitralis***

4. Costa with series of dots; cornuti absent …… ***Glyphodes canthusalis***

4a. Costa without dots; cornuti present

5. Ductus bursae uniform tube-like; ductus seminalis originates at antrum ....***Glyphodes pulverulentalis***

5a. Ductus bursae constricted and enlarged; ductus seminalis originate below antrum …… ..... ***Glyphodes caesalis***

Hampson (1896) described 48 species in the genus *Glyphodes* in the Fauna of India. Many species originally described in *Glyphodes* are currently referable under several genera such as *Palpita* Hübner, 1808, *Parotis* Hübner, 1831, *Stemorrhages* Lederer, 1863, *Arthroschista* Hampson, 1893 (Mathew, 2006). Sutrisno (2002, 2003) studied the phylogenetic relationship among the Australian *Glyphodes* group and 17 genera which are morphologically similar. According to Sutrisno, the genus *Glyphodes* is not a monophyletic group and the genitalia characters of *G. bivitralis* and *G. stolalis* are also confirmed with his study. *Glyphodes pulverulentalis* is a serious pest of mulberry in Karnataka, Andra Pradesh and Tamil Nadu (Geetha Bai *et al.*, 1997; Samuthiravelu *et al*., 2010; Rahmathulla *et al*., 2011). The pest is also reported in different locations of India *viz*., Nagaland (Gupta, 1994), Jammu (Sharma and Tara, 1985), Kashmir (Dar,1993) and Punjab (Mavi *et al*., 1996). Fletcher (1914) reported *G. caesalis* as a pest of jackfruit in Karnataka and Maharashtra. It is also recorded from Assam, Sikkim, Bihar, Uttar Pradesh, Andhra Pradesh and Tamil Nadu of India (Chowdhury and Majid, 1954; Prarthna *et al*., 2014). Soumya *et al*. (2015) reported *G. caesalis* for the first time from Kerala. In India, Kirti and Sodhi (2001) recorded five species (*G. bicolor*, *G. caesalis*, *G. canthusalis*, *G. pulverulentalis*, *G. stolalis* and *G. zelleri*) from North-Eastern India, Rose (2001) recorded seven species (*G. actorionalis*, *G.caesalis*, *G. multilinealis*, *G. canthusalis*, *G. pulverulentalis*, *G. stolalis* and *G. zelleri*) from Assam.

Tamil Nadu is endowed with rich flora and fauna that contribute to the biodiversity. Despite *Glyphodes* being a species rich tropical genus. Only six species were recorded during the present study. The diversity of Crambidae may vary in different locations with different climatic conditions. Therefore proper collection, identification and documentation of these species provide the most reliable data for conservation and management practices.

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Morphological and molecular characterization of *Limnometra fluviorum* (Fabricius) (Hemiptera: Heteroptera: Gerridae)

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ABSTRACT: The species *Limnometra fluviorum* (Fabricius) 1798 is redescribed with morphological and molecular taxonomic approach. The molecular work involving CO1amplification is the first time approach for this species from India. The diagnostic characters with measurements of different body parts are provided. © 2017 Association for Advancement of Entomology

KEY WORDS: Gerridae, *Limnometra fluviorum*, Taxonomy, DNA Barcoding

INTRODUCTION

Genus *Limnometra* Mayr, 1865 (Hemiptera: Gerridae) consisting of a group of large, black to brown colored, long legged water striders which are widely distributed and occur in a wide variety of freshwater habitats across Oriental, Malaysian, Southern and West Pacific and Northern Australia (Polhemus and Polhemus, 1997). The gerrids are predatory in nature and play a major role in the food chain of freshwater ecosystem (Thirumalai, 2002). The genus *Limnometra* Mayr was attracted the attention of taxonomists. The major revisionary work on this genus was carried out by Hungerford and Matsuda (1958) revised the genus, Nieser and Chen (1992) worked on Indo-Australian fauna, Andersen and Weir (1997) documented on Australian fauna, Polhmeus and Polhemus (1997) studied on fauna of New guinea. Zettel (2001) described one new species from India and Thirumalai (1986, 2002) provided the distribution of the genus *Limnometra* across the Indian States, which shows only three species from India. All these scientists described species of *Limnometra* through morpho-taxonomy especially based on male genitalia and mesofemoral armature. The detailed morphological characteristics with the measurements of different body parts of this species were not presented so far after the taxonomic work of Hungerford and Matsuda (1958). Furthermore, if only females and nymphs are found, they are not identifiable using morphological keys. In order to solve this difficulty, DNA barcoding technique through amplification of mitochondrial gene Cytochrome c oxidase I (COI) will be very useful to identify species irrespective of its stage and rapid, simple and widely applicable now a days (Raupach *et al.*, 2014).

Cytochrome c oxidase I (COI) of the mitochondrial DNA has been widely used as popular marker for identification and understanding the evolutionary

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relationships among the insects. Mitochondrial gene COI can be considered as the core of a universal bio-identification system for animals. COI has been already proved to be a standardized DNA Barcode for identification of Heteroptera (Damgaard *et al*., 2000; Park *et al.*, 2011; Raupach *et al.*, 2014) species regardless of their developmental stage. Keeping the above in view, an integrated approach using morpho-taxonomy and DNA barcoding was used to characterize *Limnometra fluviorum* (Fabricius) from India.

MATERIALS AND METHODS

Collection and Preservation of samples

During the recent entomological survey to the State of Odisha, India, samples were collected using net from the streams flowing across Satkosia Tiger Reserve of Angul District of Odisha. The collected samples were preserved in absolute alcohol. The samples were identified using binocular microscope Leica M205A and photographed using the same. The measurements of different body parts were taken in millimeters (mm). The male genitalia was dissected and cleared in 10% KOH. The genitalia was mounted on glass slide using Canada Balsam. The samples were stored at -20^{o} C for molecular studies.

Molecular analysis: DNA isolation, PCR amplification and sequencing

Identified gerrid samples stored in absolute alcohol were first washed well with distilled water followed by dissection of the fore leg. Fore leg samples were homogenized using a micro pestle in 50 µl of lysis buffer and treated with proteinase K at 37°C. The genomic DNA was isolated using a DNA extraction kit (QIAGEN DNeasy blood and tissue kit Cat. 69504, Germany). The barcode region of COI gene was amplified using Advantage 2 Polymerase PCR kit (Takara Clontech Japan). Standard primers for amplifying the COI region such as LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' (forward) and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (reverse) were used in the PCR. The PCR reaction was performed using a thermal cycler (c-1000 Thermal Cycler, Biorad Laboratories, California) with the following cycle conditions: 95°C for 5 minutes, followed by 34 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for one minute and a final extension at 72°C for 5 minutes. Amplified products were separated on 1.2% agarose. Gels were stained using ethidium bromide (1%). 1 kbp DNA standards were run along with the samples for reference. The PCR products were gel eluted with QIAquick Gel Extraction Kit (Cat. No. 28704, Germany) and direct sequenced using a BigDye terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster city, CA) and Applied Biosystems prism 310 Genetic Analyzer. The sequence information of individual specimens are publically available at NCBI GenBank nucleotide sequence database (Table 1) and Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert, 2007).

RESULTS AND DISCUSSION

Morphological identification of *Limnometra fluviorum* (Fabricius) (Fig.1-9)

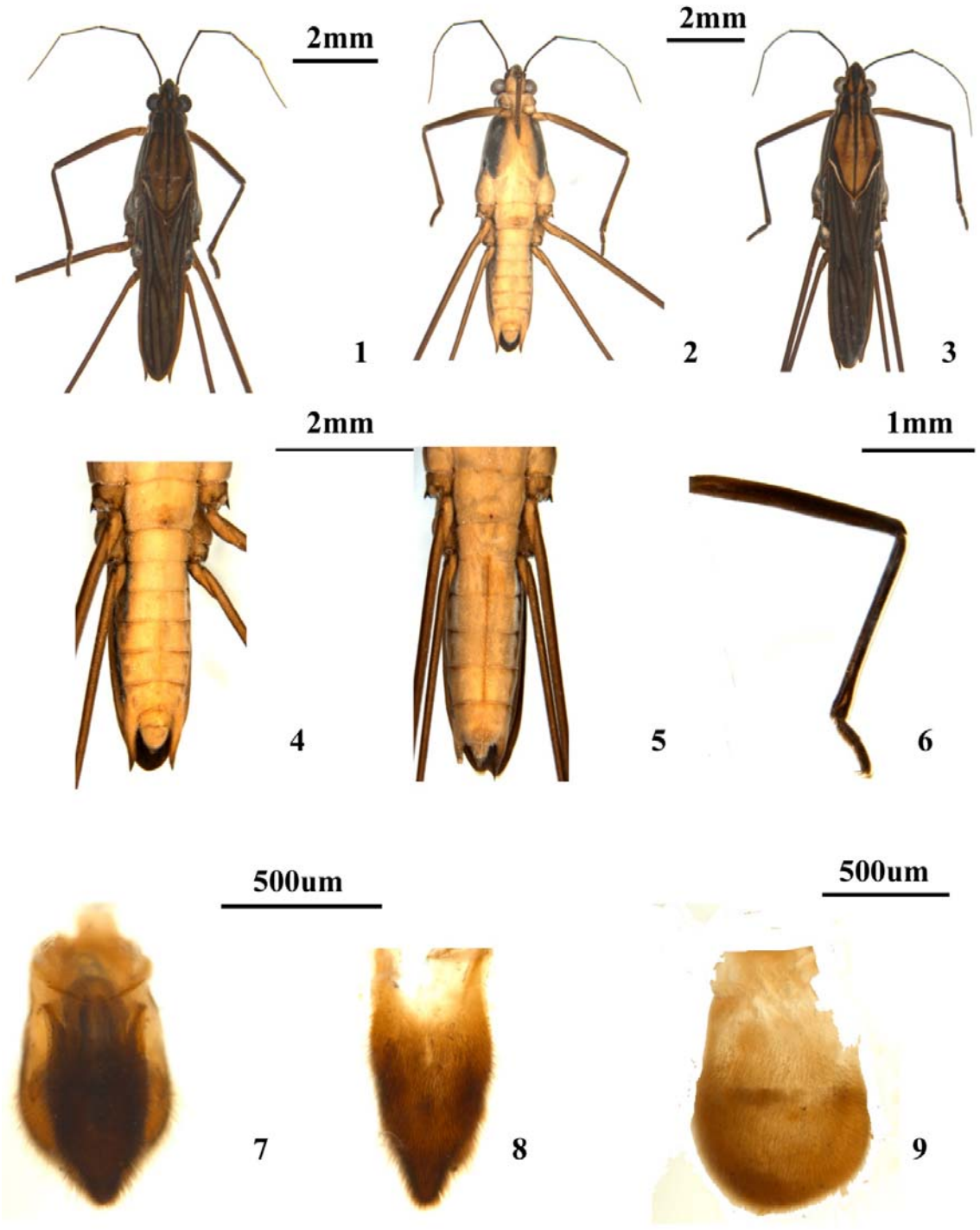
Material examined: 2M, 3F, from Katarang Nala, Satkosia Tiger Reserve, Angul District, Odisha, 21.706084N, 86.447282E, alt: 759 ft; date: 22.12.2015; coll: Dr. K. Valaramathi, Accession no. KX300087.

Description:

Size: Male attains a body length of 10.12 mm to 10.60mm. Female generally attains a length of 10.71mm to 11.54mm.

Table 1. Details of sample used in the study

Species	Sample ID	GenBank Accession	BIN number
L. fluviorum	AIN19H001	KX300087	BOLD:ACM1305



1. **Dorsal view of macropterous male**
2. **Ventral view of male**
3. **Dorsal view of macropterous female**
4. **Male abdominal sternite**
5. **Female abdominal sternite**
6. **Male foreleg**
7. **Dissected genital segment**
8. **Proctiger of male**
9. **Pygophore of male**

Colour: Usually dark brown to black with black markings. Antennae, rostrum and legs dark brown. Eyes black. Head black with typical orange markings. Pronotum brown with black longitudinal marking, extending up to metanotum. Connexival spines dark brown. Wings dark brown with prominent black markings. Ventrally yellowish. Fore wings dark brownish with black venation.

Structural characteristics: Head length 0.69 and width 0.78. Length of antennomere 1st to 4th: 2.01, 1.41, 1.45, 1.91. Eye length 0.59 and width 0.43. Interocular width 0.75. Humeral length 3.24 and width 1.87. Rostrum 2.53 in length. Male fore femur slender and unarmed, length 3.02 and width 0.33. Meso coxa with distinct spine like projection on its rear margin. Length of abdomen in male 4.22 and width 1.45, whereas in female, length of abdomen 6.15 and width 1.64. Female sternite with a distinct median ridge; however in male the median ridge is not so prominent. Connexival spines long and curved in both sexes. Length of wings 7.64.

Male genital segment: Male genital segment little elongated and 0.89 in length and 0.53 in width. Male proctiger (Fig.8) long and 'v' shaped, narrow, hairy. Pygophore (Fig. 9) truncated towards apex and broad, with hairs. Paramere vestigial. Endosomal sclerite well sclerotised.

Diagnostic characters: This species can be easily identified by presence of its spine like projection on dorso-lateral rear margin of mesocoxa of mid leg. Mesopleura black, especially with large black marking.

Distribution: India (Karnataka, Kerala, Maharashtra, Pondicherry, Tamil Nadu, West Bengal), Philippines, Sri Lanka.

Molecular identification

The size of the COI PCR product of *L. fluviorum* was 650 bps and the sequences obtained were compared with the homologous sequences (KC880894, KC880945, KC880945) available at GenBank (https://www.ncbi.nlm.nih.gov/) and species sequences reported in BOLD databases (http://www.boldsystems.org/). The accession number and Bin number are provided in Table 1. Multiple sequence alignment of the COI showed a total of 780 bp. We found larger gaps in alignment due to the length differences in sequences. To deal with this issue, we screened alignment by using trimAL software to remove the unreliable region and obtained column which are having reliable phylogenetic signals. After refinement, we got a total of 540 bps for COI. The Barcode index numbers (BINs) analysis of COI sequences was performed using BOLD Systems v3 and all (100%) fulfilled BOLDs quality criteria (Ratnasingham and Hebert, 2007).

The study described morphological and DNA barcode of mitochondrial CO1 of *L. fluviorum* for the first time in India. Furthermore, mitochondrial CO1 sequence for this specimen would be a reference source in the GenBank and BOLD database. The study also indicates that the barcoding technique can be used effectively for identification of semi-aquatic bugs. There are still many known and unknown species of Indian semi-aquatic hemiptera, several of which are not molecularly characterized, Hence this study will be the first record and the reference for further analysis.

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Impact of yam bean genotypes on growth and development of spotted pod borer *Maruca vitrata* G. (Lepidoptera: Pyralidae)

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ABSTRACT: Impact of eight yam bean genotypes *viz;* DHP-2, DPH-11, DPH-17, DL-14, DPH-18, DPH-45, Nepali and R.M-1 on growth and development of *Maruca vitrata* G. under laboratory conditions studies indicated that, R.M-1 favours spotted pod borer in laying eggs with maximum fecundity of female moth (80 eggs per female). Length of all the five instars and larval weight was also found maximum in genotype R.M-1, showing the suitability of this genotype for the growth and development of spotted pod borer. Among the test genotypes, the total life cycle of *M. vitrata* was shorter and faster on R.M-1 showing its preference.

KEYWORDS: Spotted pod borer, *Maruca vitrata*, yam bean, genotypes

INTRODUCTION

Among tuber crops, yam bean (*Pachyrhizus erosus* L.) occupies an important place and is being widely grown in uplands of Bihar, West Bengal, Uttar Pradesh, Odisha and Assam. It is popularly known as Mishrikand, Kesaur in Bihar, Sankalu in West Bengal, Assam and Odisha. It belongs to leguminocea family and commercially propagated by seed. Yam bean crop when grown for seed purpose, its flower buds and pods are reported to be infested by a Lepidopteron pest identified as spotted pod borer, *Maruca vitrata* G., with the extent of pod damage up to 40.0 per cent in Bihar (Singh and Yadav, 2006). Besides yam bean, this pest also occurs on many other economically important grain legumes (Chandrayudu *et al*., 2005).Present study was undertaken to study growth and development of spotted pod borer on different genotypes of yam and its growth index.

MATERIALS AND METHODS

Biology of *M. vitrata* was studied in the laboratory conditions during October to November, 2009-10 and 2010-11 in the Department of Entomology, T.C.A., Dholi. The initial culture of *M. vitrata* was developed in laboratory (Sunitha *et al*., 2008) by collecting two hundred larvae from field on unsprayed yam bean crop. These larvae were utilised for maintaining the mass culture of *M. vitrata*, the larvae were reared on separate clean and sterilized glass jar of 30cm diameter and 10cm height on flowers and pods of yam bean. The open end of jars was covered with muslin cloth for proper aeration and tight with rubber band. As soon as larvae started to pupate these were transferred to another petriplates containing flowers and leaves of yam bean at the bottom. After getting population, one pair of pupae consisting male and female which were sexually differentiated on the basis of

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morphological traits (genital pore situated ventrally on 9th abdominal segments in male and 8th abdominal segment in case of female) were kept separately in glass jar for emergence of adults. Each glass jar contains flower shoot, pods and leaves of one genotypes of yam bean and all the genotypes along with pupae were replicated thrice. Similarly 10 pupae were kept in a separate glass jar and covered with muslin cloth for emergence of adult. Eight pair of freshly emerged adult moth were transferred into eight separate glass jar with filter paper at bottom and each jar contained flower, buds, leaves and pods of each genotypes for oviposition. A cotton swab soaked in 0.2 per cent sugar solution was provided in each glass jar for adult feeding. Eggs laid were collected from young leaves, flowers and pods and allowed for hatching. The newly hatched larvae were transferred into fresh container with tender buds, flowers and pods of further larval development. The biological parameters such as pre-oviposition, oviposition, adult longevity, incubation period, larval length (instar wise), larval duration, larval weight, pre-pupal period, pupal period, sex ratio, egg hatchability and adult feeding were recorded. The impact of different genotypes on growth and development of *M. vitrata* was studied in consideration of earlier reports of Chandrayudu *et al*. (2005) by analyzing the data on different biological parameters following Completely Randomize Design and making stage-wise comparison.

RESULTS AND DISCUSSION

Pre-oviposition period: On perusal of pooled mean data of two years, considerably shorter pre-oviposition period (1.44 days) was recorded on R.M-1 while it was maximum (1.82 days) on DPH-2 (Table-1). The pre-oviposition period on the genotypes like- Nepali, DPH-45 and DPH-18 was1.50, 1.61 and 1.71 days, respectively which being higher than that on R.M-1. Remaining genotypes DL-14, DPH-17 and DPH-11 recorded 1.77, 1.79 and 1.80 days, respectively which were statistically at par.

Oviposition: Oviposition period ranged from 3.48 to 3.95 days with minimum and maximum being on DPH-2 and R.M-1, respectively (Table-1). It was shorter on R.M-1 and longer on DPH-2 showing impact of genotypes. No work seems to have been done earlier on these aspects in relation to yam bean genotypes but the reports of some workers who studied the effect of certain host plants on this biological parameter of *M. vitrata* supported the present finding (Chinnabhai *et. al.* 2002; Ghorpade *et. al.* 2006; Bindu and Jhala, 2007).

Number of eggs laid by *M. vitrata* varied considerably depending upon the type of genotypes used on its larval food. Maximum number of eggs (80.00) per female was laid when the genotypes R.M-1 was used as food for the larvae, while it was minimum (65.40) in case of females reared on DPH-2. Significant differences were observed in number of eggs laid per female from the adults reared on genotypes *viz;* DPH-11, DPH-17, DL-14, DPH-18, DPH-45 and Nepali were recorded be 68.30, 69.30, 70.60, 71.70, 72.30 and 74.60, respectively. Among the genotypes, R.M-1 proved most suitable for the reproduction followed by Nepali. Remaining genotypes DPH-45, DPH-18, DL-14, DPH-17 and DPH-11 occupied the position in ascending order and were statistically on par. Eggs were laid in batches of two to seven glued to the surface of flowers and pods. The freshly laid eggs were pale yellow or white in colour later develop in darkish towards the centre of eggs.

Although, there were evidences in literature to show the fecundity of this insect got influenced by the type of host plants used as larval food (Ghorpade *et. al.* 2006; Bhagwat *et. al.* 2006; Bindu and Jhala, 2007 and Sonnune *et. al*; 2010); however, the present findings are first one so far on the effect of yam bean genotypes on oviposition capacity of *M. vitrata* concerned.

Incubation period: The duration of egg period on different genotypes exhibited significant variation ranging from 2.50 to 4.00 days, the shortest and longest being on R.M-1 and DPH-2, respectively (Table-1). In Nepali, DPH-45, DPH-18, DL-14, DPH-17 and DPH-11 it was 2.60, 2.70, 2.90, 3.20, 3.40 and 3.90 days respectively. The incubation period recorded on R.M-1, Nepali, DPH-45, DPH-

18, did not differ from each other statistically whereas, the genotypes DL-14, DPH-17 and DPH-11 were close to DPH-2. Chandrayudu *et al*. (2005), Bindu and Jhala (2007) and Sonnune *et al.*(2010) reported that the incubation period of this pest greatly influenced by types of food plants used for its rearing.

The viability of eggs registered a significant variation and it was found to be lowest and highest on the genotypes DPH-2 (51.83%) and R.M-1 (72.39%). The viability of eggs laid on Nepali (70.25%), DPH-45 (64.06%), DPH-18 (62.22%) and DL-14 (59.91%) proved significantly higher than that on DPH-17 (53.18%) and DPH-11 (52.87%) which did not differ significantly from DPH-2 (51.83%) but proved significantly inferior to R.M-1 (72.39%) (Table 1).

From the above results, it is concluded that R.M-1 and DPH-2 proved most and least favourable genotypes, respectively for egg viability (Hatchability) of *M. vitrata*. Bhagwat *et al.* (2006) who recorded higher hatchability on pigeon pea genotypes ICPL-90036-MI-2 followed by ICPL-90011, while lowest on MFG 537-MI-2-M5. Likewise, Ghorpade *et al*. (2006), Bindu and Jhala (2007) and Sonnune *et al*. (2010) also recorded variation in egg viability of *M. vitrata* when reared on different host plants.All these studies corroborate the present findings.

Larval development: The length first instar larvae white in colour with brownish head ranged 1.40 to 1.80 mm on different yam bean genotypes and it was significantly longer in R.M-1 (1.80 mm), while shotest (1.40 mm) on DPH-2. There were no significant differences among Nepali, DPH-45 and DPH-18 (1.75 mm, 1.74 mm and 1.73 mm respectively). On DL-14, DPH-17 and DPH-11 it was recorded as 1.69mm, 1.56mm, 1.46mm respectively (Table-1).

The second instar larvae were recognized by creamy white in colour with dark patches on the body. Length of second instar larvae ranged from 5.75-6.33 mm on different yam bean genotypes and it was significantly longer on R.M-1 while shortest on DPH-2. In Nepali, DPH-45, DPH-18, DL-14, DPH-17 and DPH-11 a mean value of 6.28, 6.25, 6.20, 6.17, 5.91 and 5.80 mm respectively were recorded.

The third instar larvae were recognized from other instar by the presence of prominent dark patches on the body and creamy white in colour. The length of third instar varied from 7.90mm to 8.46mm. Maximum (8.46 mm) was recorded on R.M-1 and minimum (7.90 mm) on DPH-2. The mean length of larvae recorded on Nepali, DPH-45, DPH-18 and DL-14 was around 8.44, 8.41, 8.40 and 8.37 mm respectively with non-significant difference among them. The remaining genotypes occupied intermediate position.

The length of fourth instar larvae revealed that significantly higher (11.36 mm) when they were reared on the genotype R.M-1, while lowest larval length (10.81 mm) was recorded on DPH-2 which was at par with that on DPH-11 (10.86 mm). No significant differences in it were observed when larvae reared on Nepali and DPH-45 with its mean value of 11.29mm and 11.25 mm, respectively. On the other hand, DPH-18 and DL-14 showed almost similar effect on the length of developing larvae with mean value of 11.24 and 11.20 mm, respectively followed by DPH-17 (11.04 mm) (Table 1).

The fifth instar larvae were brownish in colour with dark brown head and absence of body spots. Significantly longer length (17.39 mm) was recorded when larvae were reared on R.M-1 followed by Nepali (17.33 mm) and lowest in DPH-2 (16.72). There was no significant difference in respect of larval length when larvae were reared on DPH-45 (17.30 mm), DPH-18 (17.29mm) and DL-14 (17.26mm) and remaining two genotypes DPH-17 and DPH-11 occupied sixth (17.11mm) and seventh (16.81mm) position. The present findings got support from the reports of Ghorpade *et al.* (2006), Bindu and Jhala (2007) and Sonnune *et al.* (2010) who studied the impact of various host plants on this pest.

Total larval duration of *M. vitrata* when reared on different yam bean genotypes exhibited significant difference. Shortest larval period 10.80 days was recorded on R.M-1 which was statistically at par

with Nepali (11.00), DPH-45 (11.50) and DPH-18 (11.80). On DL-14, DPH-17, DPH-11 and DPH-2 it was 12.00, 12.30, 12.60 and 13.00 days respectively. Statistically the yam bean genotypes DL-14, DPH-17, DPH-11 and DPH-2 were at par with each other in influencing the rate of larval development of the pest under study. None of the references was found on effect of different yam bean genotypes on larval duration of *M. vitrata*. However, a number of earlier workers reported impact of different host plants other than yam bean on larval duration of *M. vitrata* (Chinnabhai *et al.*, 2002; Chandrayudu *et al*., 2005 and Sonune *et al.*, 2010).

Weight attained by full grown larvae of *M. vitrata* differed significantly among different genotypes of yam bean. Pooled mean data of two years clearly revealed that significantly higher larval weight (75.00 mg) was recorded when the genotype R.M-1 was used as food while the lowest larval weight (56.30 mg) was recorded on DPH-2. Larvae reared on Nepali and DPH-45 weighed 71.90 and 66.20 mg, thus occupying second and third position, respectively. On remaining genotypes DPH-18, DL-14, DPH-17 and DPH-11, the larval weight of *M. vitrata* was recorded to be 63.20, 62.40, 61.40 and 58.10 mg respectively. No work have been reported so far on the effect of yam bean genotypes on larval growth and development of *M. vitrata*. However, evidences are available in literature to show the differential effects of host plants other than yam bean on the larval weight (Bhagwat *et al*., 2006 and Sunitha *et al*., 2008).

The full grown larvae stopped feeding before pupation and spun transparent silken webbing around its body in which it finally transformed into pupa. The pre-pupal stage was greenish in colour. On the basis of mean of two years data presented in Table-1, it revealed that shortest pre-pupal period (1.32 days) was recorded on R.M-1 which was statistically at par to Nepali (1.34 days). The longest pre-pupal period (1.66 days) was recorded on DPH-2 which was at par to DPH-11 (1.64 days). The full grown pupa was radish brown in colour and it was observed that the pupation takes place generally on flowers and sometimes at the bottom of rearing container. The duration of pupa was found considerably influenced by yam bean genotypes on which its larvae were reared. On R.M-1 it was shortest (6.50 days), while longer (7.90 days) on DPH-2. On the remaining genotypes Nepali, DPH-45, DPH-18, DL-14, DPH-17 and DPH-11, the mean pupal periods were 6.70, 6.90, 7.00, 7.10, 7.40 and 7.70 days, respectively. While pupal weight of male and female pupae ranged from 27.86 to 33.34 and 28.67 to 35.50 mg with minimum and maximum being on DPH-2 and R.M-1 respectively. No work seems to have been reported earlier on this aspect with particular reference to yam bean genotypes. However, the present findings got a good support from the reports of earlier workers (Bhagwat *et. al.* 2006; Sonnune *et. al*; 2010) who recorded variation in pupal survival of this insect in response to different host plants used as its larval feeding.

Sex ratio: Females outnumbered males irrespective of genotypes of yam bean. On the basis of means of two years data it revealed that the sex ratios of male to female were worked out to be 1:1.3, 1:1.3, 1:1.3, 1:1.3, 1:1.4, 1:1.4, 1:1.4 and 1:1.5 on DPH-2, DPH-11, DPH-17, DL-14, DPH-18, DPH-45, Nepali and R.M-1 respectively (Table-1).

Longevity: Adult longevity of either sex varied significantly on different genotypes under test used as larval food. Both male and female adults lived for shorter period on DPH-2, while longevity of both the sexes was more on R.M-1. No work seems to have been done earlier on this aspect in relation to yam bean genotypes. However, considerable variations in adult longevity of either sexes of *M. vitrata* on different host plants other than yam bean which served as larval food were recorded by various workers (Ghorpade *et al*., 2006; Bhagwat *et al.*, 2006 and Sunitha *et al*., 2010).

Total life cycle: It was shorter on the yam bean genotype R.M-1(29.36 and 30.86 days in case of male and female respectively). It was recorded as 29.62, 30.36, 30.90, 31.40, 32.08, 32.45 and 33.39 days in case of male; while 31.21, 31.76, 32.40, 32.91, 33.28, 34.05 and 34.69 days in case of female

Table 1. Impact of yam bean genotypes on growth and developement of *Maruca vitrata* G. (pooled mean - 2009-10 & 2010-11)

Parameters	DPH-45	DL-14	DPH-17	R.M-1	DPH-11	Nepali	DPH-18	DPH-2	SEm (+)	CD (P=0.05)
Pre-oviposition (days)	1.61	1.77	1.79	1.44	1.80	1.50	1.71	1.82	0.01	0.03
Ovipostion period (days)	3.87	3.82	3.66	3.95	3.51	3.88	3.84	3.48	0.01	0.04
No. of Egg laid/Female	72.30	70.60	69.30	80.00	68.30	74.60	71.70	65.40	0.39	1.07
Incubation period (days)	2.70	3.20	3.40	2.50	3.90	2.60	2.90	4.00	0.22	0.62
Egg hatchability (%)	64.06	59.91	53.18	72.39	52.87	70.25	62.22	51.83	0.46	12.70
Length of larvae (mm)										
1st instar	1.74	1.69	1.56	1.80	1.46	1.75	1.73	1.40	0.01	0.02
2nd instar	6.25	6.17	5.91	6.33	5.80	6.28	6.20	5.75	0.01	0.02
3rd instar	8.41	8.37	8.23	8.46	8.11	8.44	8.40	7.90	0.01	0.04
4th instar	11.25	11.20	11.04	11.36	10.86	11.29	11.24	10.81	0.01	0.04
5th instar	17.30	17.26	17.11	17.39	16.81	17.33	17.29	16.72	0.01	0.03
Larval period (days)	11.50	12.00	12.30	10.80	12.60	11.00	11.50	13.00	0.39	1.07
Full grown larval weight (mg)	66.20	62.40	61.40	75.00	58.10	71.90	63.20	56.30	0.36	0.99
Pre-pupal period (days)	1.40	1.60	1.62	1.32	1.64	1.34	1.56	1.66	0.01	0.03
Pupal period (days)	6.90	7.10	7.40	6.50	7.70	6.70	7.00	7.90	0.024	0.67
Pupal weight (mg)										
Male pupa (mg)	30.17	28.92	28.29	33.34	28.10	30.79	29.25	27.86	0.57	1.60
Female pupa (mg)	32.14	30.34	29.17	35.50	28.92	34.23	30.95	28.67	0.82	1.31
Adult emergence (%)	76.67	73.33	71.67	81.67	68.33	78.33	76.67	71.67	3.12	9.35
Sex ratio (M/F)	1:1.4	1:1.3	1:1.3	1:1.5	1:1.3	1:1.4	1:1.4	1:1.3	-	-
Adult longevity (days)										
(a) Male	4.00	3.70	3.70	4.30	3.60	4.10	3.80	3.40	0.19	0.52
(b) Female	5.40	5.20	4.90	5.80	4.70	5.70	5.30	4.70	0.21	0.58
Total life cycle-Male	30.36	31.41	32.08	29.36	32.45	29.62	30.90	33.39	-	-
Total life cycle -Female	31.76	32.91	33.28	30.86	34.05	31.21	32.40	34.69	-	-
Growth index	5.24	4.21	3.81	7.17	3.47	6.12	4.28	3.11	-	-

on Nepali, DPH-45, DPH-18, DL-14, DPH-17, DPH-11 and DPH-2, respectively. No work seems to have been reported earlier to ascertain the differential effect in any of the yam bean genotypes on the total life cycle of *M. vitrata*. Bindu and Jhala (2007) reported that the total life cycle of male and female varied from 27.20-30.00 days and 29.36-31.17 days respectively on different host plants. Similar results were obtained by Chandrayudu *et al.* (2005), Ghorpade *et al.(*2006) and Sonnune *et al.* (2010).

Growth index: It was maximum (7.17) on R.M-1 showing its superiority for larval food over all other genotypes. It was closely followed by Nepali and DPH-45 with a growth index value of 6.12 and 5.24, respectively. Lowest growth index value (3.11) was recorded on the genotypes DPH-2 indicating less preferred genotype for the larvae of *M. vitrata*.

The present findings thus amply demonstrate that the R.M-1 and DPH-2 proved to be the most and least preferred food plants respectively for the larvae of *M. vitrata* as reflected by highest and shortest growth index value. No information seems to be available in literature on the relationship between the yam bean genotypes and larval growth of *M. vitrata*. However Ramasubramanian and Babu (1989) and Bindu and Jhala (2007) studies on other host plants support the present findings. On the basis of results for the impact of growth and development of spotted pod borer on different genotypes of yam bean *viz;* DPH-45, DL-14, DPH-17, R.M-1, DPH-11, Nepali, DPH-18, DPH-2, it can be concluded that R.M-1 was found to be most preferred host while DPH-2 to be the least preferred host for growth and development of spotted pod borer, *M. vitrata*.

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Illustrated redescription of two large coreid bugs from Assam including *Schroederia feana* (Distant, 1902) as the first record for India (Hemiptera, Heteroptera, Coreidae, Coreinae, Mictini)

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ABSTRACT: *Schroederia feana* (Distant, 1902) is recorded for the first time from the present Indian Territory and redescribed based on male specimen from Assam, India. In addition, *Prionolomia gigas* Distant, 1879 is redescribed based on male and female specimens from the same locality.

KEYWORDS: Coreidae, Mictini , Taxonomy, *Schroederia feana, Prionolomia gigas*

INTRODUCTION

During a brief survey onthe private premises of Makunda Christian Hospital, Karimganj District, Assam, two interesting and large Coreidae bugs were collected. One was identified as *Schroederia feana* (Distant, 1902) and the other as *Prionolomia gigas* Distant, 1879, based on keys in Distant (1902). Generic characters and nomenclatural changes were confirmed using keys and descriptions in O'Shea and Schaefer (1980). *S. feana* was described as *Derepteryx feana* by Distant (1902) from Tenasserim, Thagata, in the present day Myanmar,and its transfer to the genus *Schroederia* has been discussed, with history, by O'Shea and Schaefer (1980). Dispons (1962) also studied *D. feana* but he had placed it in another genus *Axinepteryx*, according to him the distribution of this species is : 'Burma, Thailand, Sumatra and Borneo' and O'Shea and Schafer, who placed it in *Schroederia*, gave distribution as 'SE Asia, Indonesia'.Recent list of coreids of India (Prabakar, 2013) does not include *S. feana* from any part of India; its occurrence in Assam is therefore the first record of this coreid from India.

Prionolomia gigas, one of the largest coreid, is known to be present in Assam (Distant, 1879); Breddin (1900) described the female of this species, also from Assam. In spite of the fact that both bugs are quite large, these are not well illustrated or redescribed before. Revision of the tribe Mictini

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by O'Shea and Schaefer (1980), which includes both these species,gives only generic diagnosis, with the list of included species, and a few small-sized line drawings. Dispons (1962) however, described generic characters and also gavea key to all the species of *Prionolomia* as well as illustrated some major characters.

None of these previous works include photographic images of dorsal and ventral habitus of important diagnostic characters. To help in identification of these two bugs, especially for students and biodiversity surveyors, we are providing photographically illustrated redescription of both these species.

MATERIALS AND METHODS

Methods of morphometry and preparation of illustrations are described earlier (Kulkarni and Ghate, 2016). Synonyms are given by O'Shea and Schaefer (1980) and hence not reiterated here. Since there are three different spellings of *Schroederia* in the above paper, we confirmed the correct spelling in the original description (Schmidt, 1911) and the Coreoidea website was also checked for all species' names mentioned here (Coreoidea SF Team. *Coreoidea Species File Online*. Version 5.0 [Retrieval date May 26, 2016] http://Coreoidea.SpeciesFile.org.

RESULTS

***Schroederia* Schmidt, 1911**
***Schroederia feana* (Distant, 1902)**

Material examined:1 ♂, India, Assam, Karimganj District, Bazaricherra, Makunda Christian Hospital Campus, on foliage, 16.iv. 2016, Rejoice Gassah and Vijay Anand Ismavel.

Measurements (in mm): Total length 33.5 mm measured from tip of abdomen to the tip of head (measured ventrally).

Length of antennal segments: I 10; II 7, III 6; IV 6.5. Length of fore femur 10, fore tibia 8, fore tarsus 5.Length of mid femur 10,mid tibia 9, mid tarsus 4.5.Length of hind femur 14, hind tibia 15,hind tarsus 4.Body breadth at scutellum 9; maximum distance between inner margin of pronotal expansion 17; pronotal expansion length 11.

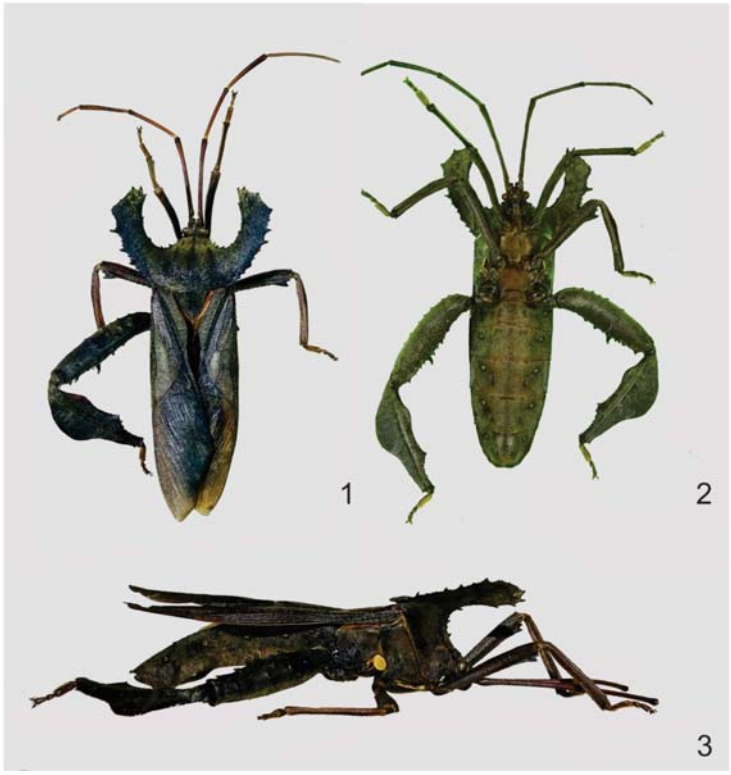
Redescription:

Colouration: Robust, moderately elongate bug, with bizarre process on pronotum; colour dark, brown to blackish. Head with antennae dark brown to blackish, pale ochraceous vertical band behind ocellus. Thorax with pronotum showing median dark line and similar but shorter line on each side. Prosternum dark brown, mesosternum ochraceous with brown band on either sides of labium, metasternum reddish brown. Abdomen dorsally reddish ochraceous with scattered pale areas; abdomen ventrally reddish brown on disc, laterally slightly darker; spiracles large, with white ring. Inner part of mid-coxae ochraceous (Figs. 1-3).

Morphology:

Head quadrate; antenniferous tubercles prominent, projecting in front beyond clypeus;eyes large, semi-globular;ocelli closer to eyes than to each other; shallow transverse sulcus at level of each ocellus; postocular tubercles small but distinct. Entire dorsal surface of head with very fine, moderately long, colourless setae. Antennae robust, hirsute, slightly shorter than body; first segment longest, second and third subequal, covered densely with long black and short colourless setae. Head underneath less setose; labium stout and long, reaching mid coxae,first segment stout, second and third less thick, second segment longest. Clypeus slightly depressed below mandibular plates which are oblique and oval, both seen only in frontal view of head, below projecting antenniferous tubercles (Figs. 4-6).

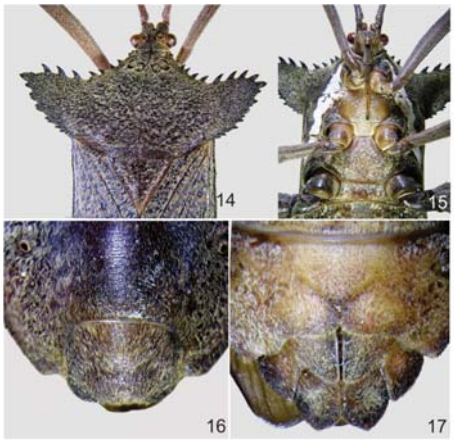
Thorax: Pronotum strongly declivous, with thin median carina, rugulose punctate on disk, with scattered granules and very fine golden setae; shape bizarre. Posterior 1/3rd of lateral margin produced laterally and anteriorly into wing-like expansions; lateral border in front of this expansion with strong, long and small spines,these spines continued along entire border ofexpansion; outer



Figures 1-3: *Schroederia feana*. 1: Habitus, dorsal; 2: Habitus, ventral; 3: Habitus, lateral.

Figures 4-7: *Schroederia feana*. 4: Details of pronotum, dorsal; 5:Details of Pronotum, ventral; 6: Details of pronotal lateral margin, dorsal; 7: Details of pronotal wing-like expansion, dorsal.

Figure 8-9 *Schroederia feana*.
8: Scutellum; 9:Male, abdomen ventral view with pygophore in situ.



Figures 14-17: *Prionolomia gigas*. 14:Details of head and pronotum, dorsal; 15: Details of head and sterna, ventral, note lateral ochraceous band; 16:Male ventral view showingpygophore in situ; 17: Female terminalia in situ, ventral view.

Figures 10-13: *Prionolomia gigas*. 10: Male, habitus, dorsal; 11: Male, habitus, ventral; 12: Female, habitus, dorsal; 13: Female, habitus, ventral.

border of expansion with strong and long spines,apical lateral region more or less truncate on outside, with two inner strong spines (Fig. 7); anterior margin of pronotum straight, anterior angles of pronotum with broad tubercle just behind collar, posterior margin over scutellum more or less straight;posterior border in front of scutellum setose with very fine granules;lateral area with spiny tubercles. Prosterum narrow, with median sulcus, its posterior tip arrow-like, lateral parts of prosternum smooth, with scattered fine punctures; underside of anterior expansion of pronotum concave, possessing scattered granules and fine folds; mesosternum more or less smooth, finely setose, mesosternal process tongue-like, squarish, projecting posteriorly between mid coxae to meet anterior truncate projection of metasternum; metasternum with fine granules and setae, its lateral extension continued as anterior border to scent gland. Scent gland prominent with a large anterior disc and a very small posterior disc on either side of ostiole.

Scutellum almost as long as broad, triangular, dark brown, transversely wrinkled in posterior half; apically ochraceous (Fig. 8).

Hemelytra long, clavus densely punctured and covered with golden setae; corium identical to clavus with veins dark and raised distinctly; membrane dark brown with sparse golden setae and with many, longitudinal parallel veins.

Leg moderately robust; hind legs conspicuously incrassate;forefemur slightly laterally compressed, tarsi of lighter colour due to dense covering of golden setae on underside of first tarsal segment. Claws black, colourless pulvilli prominent; second and third tarsal segment with dorso-median, smooth longitudinal line, rest area setose; middle femur laterally compressed, ventro-medially uniformly dentate along entire length, with one large and one small spine at apex,apically apparently dilated;tibia laterally compressed;tarsal segments dorsally dark brown with black setae, ventrally with dense golden pubescence;hind femur much incrassate, strongly dentate on dorsal and ventral surfaces and granular elsewhere;hind tibiae slightly shorter than femora, possessing dorsal and ventral expansions;ventral expansion with post medial strong triangular process,proximal part of inner margin just behind ventral spine granular, remaining border beyond triangular spine with distinct spines;distal tip with spine at right angle to dorsal and ventral margin. All legs with dense setae of three types: black, long setae, colourless pale brown setae and fine, small colourless setae.

Abdomen beneath more or less smooth with shorter, fine golden setae; trichobothrial groups very distinct; abdominal spiracles distinct, large,situated closer to anterior border than lateral border of segment;lateral abdominal border dentate, posterolateral corners with a strong tooth, especially on fourth to sixth segment. In male, pygophore not visible from dorsal side, ventrally partly visible, its ventral surface setose, with shallow median groove in posterior half. Lateral border of seventh segment distinctly dentate up to tip (Fig. 9).

***Prionolomia* Stal, 1873**
***Prionolomia gigas* Distant, 1879**

Material examined: 1 ♂, 1 ♀, India, Assam, Karimganj District, Bazaricherra, Makunda Christian Hospital Campus, on foliage, 18.iv. 2016, Rejoice Gassah and Vijay Anand Ismavel.

Redescription:

Measurements (in mm):Total length (measured from head to tip of abdomen): Male 40, female 38. **Male:** Length of antennal segments: I 10.5, II 6.5; III 6 mm; IV 10 mm; width at humeral angles 19; length of hemelytra 30; length of fore femur 11, fore tibia 9,fore tarsus 5; length of mid femur 13,mid tibia 11, mid tarsus 5; length of hind femur 16, hind tibia 16, hind tarsus 6.

Habitus and Coloration: Robust bug. Male dorsally dark brown, stout; ventrally paler with few scattered dark brown areas.Antennae overall brown, second and third segment darker apically, fourth segment much paler, almost yellow in basal part, slightly

darker beyond middle. Fore and mid legs light brown, and hind legs darker. Thoracic sterna laterally with broad ochraceous band. Scutellum dark brown but its apex ochraceous. Hemelytra dark brown; membrane with several longitudinal veins; hind wings long with transparent pale brown coloration; veins dark brown, raised. Abdominal tergites distinctly pink red, sterna a mixture of pale brown and yellow brown (Figs. 10-11). Female identical in coloration to male but dorsally as well as ventrally much paler (Figs. 12-13).

Morphology:

Male: Head quadrate, finely setose dorsally; eyes large, globular;ocelli prominent, pink with black ring on inner margin, closer to eye than to each other, preocellar groove deep, prominent. Antenniferous tubercles prominent, projecting in front of clypeus; antennae moderately stout, segmentI longest and thick, II and III sub-equal, IV longer than second; III and IV subequal; all segments cylindrical, except fourth which is slightly flatter; head beneath finely setose; labium moderately long, stout, reaching anterior margin of midcoxae; bucculae distinct, pale coloured; clypeus and mandibular plates oblong oval, sloping, visible clearly in frontal view only.

Thorax: Pronotum appearing almost triangular due to laterally produced humeral region and very narrow anterior margin behind head; area in front of lateral projection strongly sloping,area behind more or less flat;anterior margin straight behind head; posterior margin straight over scutellum. Entire lateral margin of pronotum, up to tip of extended humeral angle, lined with strong, curved, black spines; posterior margin behind humeral angle also spinous, but spines shorter, rest of posterior margin smooth. Entire dorsal region with fine, pale brown setae. Callar area distinct, partly smooth without setae; behind callar area, entire pronotum rugulose, raised part of rugae shining and without setae; rugae indistinct on expanded portion of humerus; punctures very fine, distributed all over dorsal surface. Scutellum triangular with many transverse wrinkles and pale brown setae;lateral margin more or less straight. Prosternum moderately concave in middle and transversely depressed all around; lateral margin rugulose, punctate all over, covered with pale brown setae; prosternal process produced sharply to meet similar sharp triangular anterior process of mesosternum; mesosternum distally pale coloured, median area moderately sulcate along length, with smooth shining patch on either side on disc; rest area with pale brown setae; mesosternum posteriorly rectangular meeting similar anterior part of metasternum, borders of both processes raised above like carina; metasternum darker, finely granular and densely setose all over; posterior margin gently concave. Lateral to all thoracic segments (pleural region) runs a dense band of mostly white and few pale brown setae, interspersed with brown smooth spots. Scent gland large, prominent, situated more ventrally, with rounded disc anteriorly and raised tubercle posteriorly at lateral border (Figs. 14-15). Scutellum triangular, as broad as long, with fine wrinkles all over.

Hemelytra: Clavus and corium dark brown, finely punctured and covered with patches of pale brown setae; veins distinctly raised above as ridges, smooth and shining; outer (anterior) angle very long, projecting beyond half-length of membrane; membrane moderately broad, exposing part of connexivum laterally and extending just to tip of last tergite.

All legs moderately stout and hind legs very stout. Fore and mid femora laterally compressed, carinate dorsally and ventrally, ventral carina terminating as prominent, long subapical spine;small spine present in front of this large spine.A few granules also present on femur, appearing as if forming a line on inner face; femoral carina also appears finely denticulate at some places. Fore and mid tibia strongly compressed with a median carina on inner face. Tarsus well developed, segment I long and stout, claws widely separated, black with well-developed, colorless, pulvillus at base. Entire surface of fore and mid legs covered with dark and pale brown setae and shorter adpressed setae. Hind femur strongly incrassate, almost spindle-

shaped with a series of strong, black, pointed,spiny tubercles arranged in apparent rows on inner as well as outer surface (inner row single, outer almost three rows);in addition, anterior surface with fine granules all over. As a distinct feature of male, there is a strong posteriorly directed spine ventrally beyond mid length. Hind tibia dilated on both sides, its inner dilation producing a sharp spine near middle,entire ventral margin finely denticulate proximal to this spine and strongly denticulate beyond spine up to tip; dorsal expansion only setose without denticulation and gently sinuate. Tarsi and claws as in fore and mid legs.Entire hind leg also covered with adpressed pale brown setae, margined with dark brown erect setae. Ventral expansion of tibia stronger, partly granular with less setae than dorsal margin.

Abdomen: Tergites relatively smooth, sternites covered with dense patches of pale brown setae; discal region of sternites rugulose, with fine tubercles and laterally coarsely punctured. Spiracles large, spiracular rim raised above, pale coloured; spiracles situated closer to anterior margin than lateral margin of segment. Seventh sternite truncate posteriorly in front of pygophore, its discal area strongly rugulose with sparse setae, exposed part of pygophore rounded, pygophore dorsally also covered by posterior rugulose extension of seventh tergite; dorsal opening not visible (Fig. 16). Lateral margin of connexivum finely granulose, posterolateral angles of segments III-VI produced into spine of which those on IV and V are very prominent.

Female: Abdominal sternites paler and abdomen distinctly broader than male. Hind legs similar, but femur less incrassate and without long ventral spines; dorsal surface with less number of tubercles;tibia similar but ventral margin not produced into median spine and with margin much finely denticulate than in male. Scutellum, clavus and corium distinctly mottled with paler patches. All antennal segments smaller by 0.5 to 1 mm than that of male. Seventh sternite in female emarginate at posterior border; median surface raised as a triangular projection with its tip above emargination. Female genital segments setose (Fig. 17).

DISCUSSION

Schroederia feana is a very distinct and the only species under this genus. It is quite different from species of the genus *Derepteryx* White (in which Distant had originally placed this species), such as *D. grayii* White, 1839 and *D. hardwickii* White, 1839 (now *Molipteryx hardwickii*); as pointed out by O'Shea and Schaefer (1980), the shape of the pronotum is diagnostic. Although, Distant described *S. feana*, no illustration was provided; later in Fauna volume Distant (1902) illustrated *D. grayii* only. It is true that the type locality of *S. feana* is in the adjacent country and its occurrence in India is not surprising, however it has never been reported from India before. Lack of surveys in these parts of North-East India may be one of the reason. A photo of syntype of this species has been provided by *Coreoidea Species File Online*.

Prionolomia gigas is similar to *Prionolomia heros* (Fabricius, 1794), which is also known from Sylhet in the adjoining region of Assam (presently in Bangladesh), as per Distant (1902), due to lateral ochraceous band on thoracic pleura, but the latter is a smaller species; both, the description and three figures provided by Distant leave no doubt that our species is *P. gigas*, however type material of both these species must be compared in future to find out other differences. *Prionolomia heros heros* has also been illustrated on *Coreoidea Species File Online* and appears distinct. The other two species described in Distant (1902) are smaller and lack thoracic sternal band. Although known from India, this species has also not been recently recorded in literature. Since these species are identifiable by characters and illustrations provided here for the first time, genitalia characters are not detailed here, besides these have been partly given by O'Shea and Schaefer (1980). Photos of pyrophore, parameres and phallus of both species will be provided separately.

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Mortality of a common Indian grasshopper exposed to dietary arsenic

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ABSTRACT: Studies on the effect of various doses of arsenic on the life span and mortality rate of *Oxya velox* a common acridid revealed that this insect was found vulnerable even in lower dose, whereas, it could try to overcome the effect with the increasing doses of arsenic. From this point, it is important to remark that this insect may act as bioindicator of this heavy metal in this region as it was found to accumulate this metal.

KEY WORDS : *Oxya velox*, arsenic, accumulation, stress, mortality.

Metal pollution is one of the serious problem that face mankind in the twenty first century (Montaser *et al.*, 2010).Arsenic contamination is a major problem of the Gangetic plains of West Bengal and Bangladesh. This toxic metal is available naturally in the silt deposition of this region and it is widespread in this delta. Bioaccumulation of arsenic in the branchial tissue of *Sabellas pallanzanii* gave the evidence of environmental origin of this metal (Fattorini*et al.*, 2004). Due to contamination, arsenic may accumulate in soil, leads to decrease in soil fertility, at the same time can be taken up by plants, ultimately enter the food chain (Meharg and Rahman, 2003). Lindsay and Sanders (1990) explained the process of arsenic uptake and transfer in an estuarine food chain from phytoplankton to upper trophic levels. As chemical analogs, arsenate and phosphate are processed by producers and inhibit ATP synthesis and growth (Blum, 1966; Sanders and Windom, 1980). Malakar *et al.* (2009) reported that survival, adult body weight and adult life span were significantly decreased due to application of heavy metals like Hg on *Oxya fuscovittata.* Grasshoppers serve as major food source for some species especially amphibian, reptiles, birds and small mammals and are ecologically significant. Nath *et al.* (2012) studied the effect of arsenic contamination in *Gesonula punctifrons* and observed the important alteration in haemocyte counts. Available research concerning arsenic contamination suggested that birds were found to be highly adapted compare with other terrestrial animals as observed by Koch *et al.* (2005). Grasshoppers can represent an important bioindicator of heavy metal contamination. The present paper deals with the effect of arsenic on the mortality rate and life span of *Oxya velox* by exposing them to three concentrations of arsenic.

Adult *Oxya velox* (Fabricius, 1787) was collected from the field (22.4962° N, 88.6157° E), near Kolkata, West Bengal. Plastic jars of 10 liter capacity containing 5.0 cm thick sand at the bottom were taken as the rearing cage. The open portion

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of the cages was covered with nylon net in order to maintain the air supply properly. Rearing was carried out in laboratory conditions,the temperature and relative humidity were maintained 30±1°C and 80± 1% respectively. The female laid eggs in the sand. After approximately 30 days of oviposition the first instars hatched out from the eggs. 100 first instar larvae for control as well as each dose were considered separately. The first instars and their successive stages including the adult insects were also reared following the same procedure.

Conical flask of 50 ml capacity containing food plant *Oryza sativa* Lin. was placed in the rearing jar for providing food to the insects. To study the effects of As, fresh leaves of *Oryza sativa* Lin. were collected from the cultivated field in the college campus and dip in the dosed distilled water treated with0.0125 mg l^{-1} (Asd1) , 0.025 mg l^{-1} (Asd2) and 0.050 mg l^{-1} (Asd3) Sodium arsenate for twelve hours. Nymphs and adults of *Oryza sativa* were fed with dosed paddy seedlings to study the effect of As on the life span and mortality. For control group of host plants were grown in As free water (Schmidt *et al.*, 1991). Food was changed after every 24 hours.

The study revealed a variation in the *O. velox* life span. At 0.0125mg l^{-1} (Asd1) mortality rate was found highest in the 1st instar nymph followed by 5th instar male nymph, whereas it was recorded lowest in 3rd instar male nymph followed by 4th instar male nymph in the same dose of arsenic (Fig 1). Rate of mortality of 2nd instar nymph (59.72%) was higher in (Asd2) and both fifth instar male (75%) and female (66.67%) in (Asd1)in comparison to control. Study also revealed that Asd1 has significant effect on the 1st instar and 5th instar stage (Table 1), it was on fifth instar at Asd2. Whereas 0.050 mg l^{-1} (Asd3) was effective in almost all the stages of the experimental grasshopper. In the last few decades, increasing concentration of arsenic in the water in different parts of the world including West Bengal, India is becoming the threats for the fauna and flora including human being (Dey, 2005). Arsenic which was taken up by plants sequester in the root, followed by straw and grain in case of paddy (Imamul Huq, 2006). Present study confirmed that arsenic exerts a significant effect on the life stages of *O. velox*. Rate of mortality at Asd1 was highest on 1stinstar and 5thinstar male. In a similar observation, it was found that mercury showed the highest effect on the life span of *Aiolopust halassinus* in comparison to other experimental metals (Devkota and Schmidt, 2000). Whereas, Asd3 was found to affect all the instars in comparison to other two doses and the rate of mortality was almost higher than the control except the male, where recovery was observed. Whereas, some stages of the experimental grasshopper exposed to Asd1 and Asd2 showed higher mortality and that might be due to differential accumulation of arsenic in the insect body than that of Asd3, as found in *Oxya fuscovittata* treated with Cd (Malakar *et al.,* 2009). Present study also revealed that rate of mortality depends on concentration of doses applied during the time of experiment. Augustyniak and Miguls (2000) reported that cadmium transfer rate from host plant to the grasshopper body depended on exposure time. Grasshopper as primary consumer helps in the transfer of arsenic to higher trophic level more efficiently as suggested by Schimdt (1986) during the work on the biotransfer of geogenic heavy metals via the grasshoppers to higher trophic level.

The present study reveals that both the sexes are affected due to exposure of various doses of arsenic. Devkota and Schmidt (2000a) reported that both sexes could accumulate the heavy metals equally in their bodies, so it was not essential to give preference to one sex of grasshopper during biomonitoring. The study also revealed that an average rate of mortality was observed in Asd3 which was lower than other two doses, though it was higher than that of control. Thus indicating the grasshopper could overcome the toxic effect of arsenic to make a balance between growth and metabolism as was found in pesticides treated fish (Aguigwo, 2002). Most insects grow a mechanism of internal decontamination when exposed to heavy metals (Ballon-Dufrancais *et al*., 1980; Jeantet et al.,1980). Moreover, Devkota and Schimidt (1999) has been reported that *Eypropocnemis plorans*, a short horned grasshopper could tolerate higher concentration of Mercury, that is, improvement of

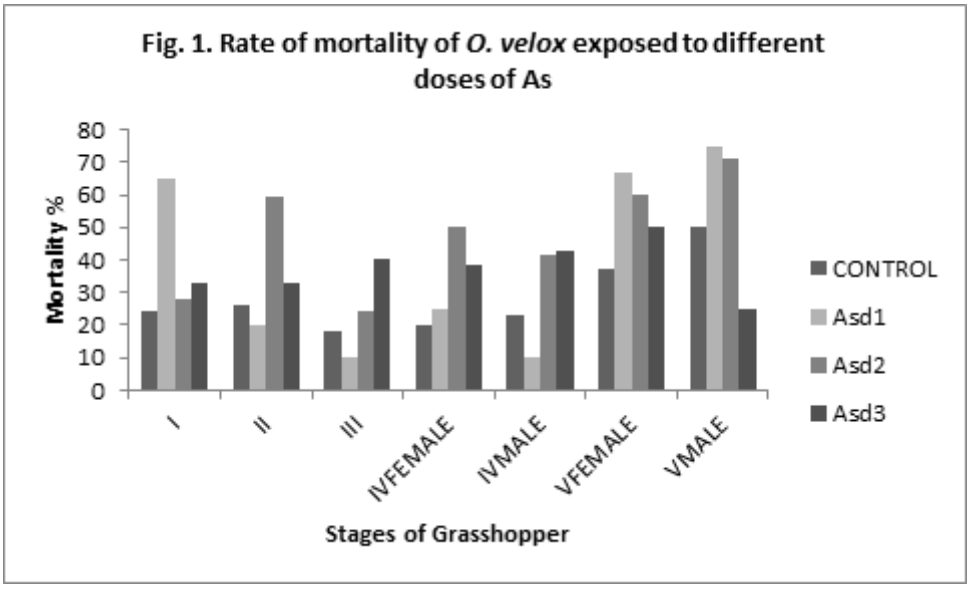

Table1. Showing the relation between Control and different doses of As

Doses	R^2	r	Regression	t-value
Control-Asd1	0.683	0.826	Y=9.62+0.966x	2.93*
Control-Asd2	0.816	0.903	Y=22.38+0.809x	4.21*
Control-Asd3	0.89	0.943	Y=16.75+0.785x	5.68*

*Significant $p<0.05$

health and growth by intaking this metal through food, indicated an affirmative effect which might be interpreted as hormesis (Hopkin, 1989). Specific heavy metals concentration dependent eclosion time of adult *Aedes aegypti* was also observed by Rayms-Keller *et al*. (1998). So the long term effect of arsenic was not only of interest in ecological direction but also for the development of the grasshopper.

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