Pest Management in Horticultural Ecosystems
Vol. 27, No.2 pp 250-257 (2021)

Antennal morphology and sexual dimorphism of the ash weevil, *Myllocerus subfasciatus* Guerin (Coleoptera: Curculionidae)

B. R. JAYANTHI MALA[1,2]*, S. V. KRISHNAMOORTHY[1], P. D. KAMALA JAYANTHI[2], N. SATHIAH[1] and N. KARTHIKA[1]

[1]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

[2]Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

***E-mail:** entjaya@gmail.com

ABSTRACT: Ash weevil, *Myllocerus subfasciatus* Guerin (Coleoptera: Curculionidae) is emerging as a major pest on brinjal. Scanning electron microscopic studies of antennal morphology and sexual dimorphism of the ash weevil, *M. subfasciatus* were carried out. The size, length of body, width of body, thorax, antenna abdomen of females was significantly larger than males (P<0.0001). The weight of females (20-70 mg) is double the weight of males (10-14 mg). The thoracic glands of males have openings on the top whereas the females are lacking such openings. We observed clear difference in the cuticular secretions structures on the male and female abdominal sternites. No difference observed in the cuticular scales, abdominal openings in the last sternites. The reproductive organs and antennal morphology of the male and female ash weevils are described.

Keywords: Ash weevil, brinjal, *Myllocerus subfasciatus*, sexual dimorphism, thoracic glands

INTRODUCTION

Brinjal or aubergine is an important vegetable crop cultivated and consumed throughout India. The production of brinjal is affected by several biotic and abiotic stresses. Several pest sand diseases cause severe loss. The ash weevil, *Myllocerus subfasciatus* Guerin (Coleoptera: Curculionidae) is an emerging threat to this corp. It was reported first time from Tamil Nadu (Nair, 1975). The adults feed singly or in groups and damage the leaves whereas, the grub stage damages the roots causing the plant to wilt and dry. Under favorable conditions it has potential to cause 100% yield loss (Shanmugam *et al*., 2021). The egg, larva, and pupal periods last for about 3–11, 3–42, and 5–7 days, respectively. More than 336 species of genus *Myllcoerus* have been described in India and worldwide including *M. discolor* Boheman*, M. diversus, M. subfasciatus* Guerin*, M. coimbatorensis* Ramamurthy & Ghai etc., (Ramamurthy and Ghai, 1988). These ash weevils are highly polyphagous in nature and are found damaging several horticultural crops, agricultural, forestry crops. The adults are dusky brownish with black markings on the elytra, morphologically both the male and female look alike except the size. At present this pest is managed through chemical pesticides application. In order to explore the semiochemicals as one of the management strategies for ash weevil, identification of these species is important along with the sex determination in adults.

MATERIALS AND METHODS

Insects

The ash weevil adults were collected from the brinjal fields of ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru, Karnataka, India (13.1348^0 N; 77.4960^0 E). The adult weevils were brought to the laboratory and maintained in plastic petri dish cage (10 cm width x 4 cm height) provisioned with brinjal leaves. These plastic dish cages were kept at ambient temperature. The adults were used for Scanning Electron Microscopic and Stereo zoommicroscopicimage analysis studies at Division of Crop Protection, ICAR-IIHR, Bengaluru.

Scanning Electron Microscopy (SEM) Images

The insect was mounted onto the copper pin having a double-sided carbon adhesive tape and were examined with a scanning electron microscope (Hitachi Tabletop TM3030) at different magnifications.

Image Analysis

For measuring the different morphological characters of male and female adults, Images were captured with a stereomicroscope (Leica M2050). A total of 10 insects each (male and female) were measured for different parameters viz., length, width of body, Head, thorax, abdomen, antenna etc., by using image analysis in Leica M2050. A total of 10 adult females and males are weighed and mean weight was calculated separately.

Statistical Analysis

Measurements of antennal segments and of each type of sensillum were compared between sexes by using the Image J software.The data on the morphological parameters were compared by t-test using Graph Pad Prism 9.0 Software.

RESULTS AND DISCUSSION

Antennal morphology

The *M. subfasciatus* is having geniculate type of antenna containing basal scape, Funicle and club segments that covered with cuticular secretions.

Scape: The scape is mounted in a socket in a more or less ring-shaped sclerotised region called the torulus, often a raised portion of the insect's head capsule. That projection on which the antenna pivots is called the antennifer. The scape is devoid of sensilla and mostly covered with scales (Fig. 2 B & C).

Funicle: The funicle is flexibly connected to the distal end of the scape and its movements in turn can be controlled by muscular connections between the scape and pedicel. "funicle" refers to the segments between the club and the scape. Funicle covered with scales (Fig.2B).

Club: flagellomeres form a club shape, they are short and funnel shape than other antennal segment. The last segment of is dome shaped with dense sensilla on it (Fig. 3 C & D).

The antennal funicle articulates at an angle to the scape originating from the rostrum. The scape and pedicel are densely covered with elongated scales and sparse distribution of sensilla was noticed. The flagellum comprised of seven club segment (Fig. 1A) There was no significant difference observed in the morphological measurements of different flagellomeres of male and female (Table.4). Similarly, there was no morphological differences were observed in the antennal sensilla, setae and scales of both the sexes. However, the length of the antenna in females (1.75 ± 0.00 mm; range: 1.73 - 1.79 mm) is slightly bigger than males (1.04 ± 0.00 m; range: 1.03 - 1.04 mm) (Table. 5).

These sensilla play an important role in signaling or communication. In both sexes, two types of sensilla was noticed viz., Sensilla Chaetica (SC) and sensilla trichodea (ST). Based on SEM images and the external morphology these two types of sensilla are subdivided into five subtypes viz., SC1, SC2, SC3,ST1, ST2.The antennomeres are distributed with three subtypes of sensilla chaetica,two subtypes of sensilla trichodea and two types of scales(Fig. 3). The club segments 1 -4 are consisting only the blunt tip sensilla trichodea (ST1), Sensilla chaetica (SC2) and two subtypes of scales elongated and ovate. Gradual increased density of SC2 was observed from club segments 1-6. Whereas, the club segments 5 and 6 are mostly covered with dense bifurcated sensilla chaetica (SC2) and Sensilla trichodea long blunt tipped (ST1) without scales (Fig. 3).

The club segment7 is dome shaped densely covered with SC1, SC3 and sparsely distributed with ST2. One row of SC2 was present at the base of the flagellomere 7. Flagellomere 7 was devoid of ST1 (Fig. 3 C& D).

Sensilla Chaetica (SC)

Sensilla cheatica are the common sensilla present on antenna and they consist of a long hair-shaft set in an obvious flexible socket. These sensilla are assumed to be mechanoreceptors involved in various activities. Different types of sensillar chaetica are distinguished based on the type of hair shaft, shape of socket, shape of grooves on the sensilla and their location (Palma *et al*, 2019).

The scanning electronmicrosopic images of *M. subfacsiatus*club segmentsare distributed with three different types of sensilla chaetica.

SC1: Sensillum chaetica 1

These are longitudinally grooved tapered pointed at the tip of the sensilla. The mean length of the sensillum is 1.67mm. The sensilla SC1 densely distributed around the club segment 7 (1050.00) (Table.2)

SC2: Sensillum chaetica 2

These sensilla are bifurcated at the tip region which are parallel to the antennal surface. The mean length of the sensillum is 1.775 mm. SC2 are densely present on the club segments 5 and 6 and one row lined up at the base of the club segment 7. Length of the SC2 sensillum is 1.775mm and the width of the two tips is 0.25mm (Tables 1 & 2).

SC3: Sensillum chaetica 3

The sensilla chaetica subtype 3 (SC3) is blunt tipped with grooves standing divergent to the antennal surface. These are present in huge numbers only on the club segment 7 (1025.00). Rest of the club segments and antennomeres are devoid of the SC3 sensilla. The length of the SC3 is 3.169mm (Tables.1 & 2).

Table 1. Description of sensillum types of *M. subfasciatus*

Sensillum type	Location	Length (mean)	Angle	Tips
SC1	Densely distributed around the circumference of club segment7.	1.671 mm	Parallel to the antennal surface	Tapering pointed tips
SC2	Distributed on club segments 1-6 and one row at the base of the flagellomere 7	1.775 mm	Parallel to the antennal surface	Bifurcated tips
SC3	Densely distributed Around the circumference of club segment 6.	3.169 mm	Parallel to the antennal surface	Blunt tip with grooves
ST1	Sparsely distributed on club segments 1-6	1.778 mm	Standing divergent to antennal surface	Elongated and blunt tips
ST2	Sparsely distributed on club segment 7	1.601mm	Standing divergent to antennal surface	Elongated and sharp tips

SC1: Sensillum chaetica type 1; SC2: Sesnsillum Chaetica type 2; SC3: Senisllum chaetica type 3; ST1: Sensillum trichoidea

Table 2. Density of antennal sensilla present on each antennomere

	Density of subtypes of antennal sensilla (Mean numbers)				
Antennomere	**SC1**	**SC2**	**SC3**	**ST1**	**ST2**
Scape	0.00	0.00	0.00	0.00	0.00
Funicle	0.00	0.00	0.00	0.00	0.00
Club segment 1	0.00	2.00	0.00	4.70	0.00
Club segment 2	0.00	10.00	0.00	7.00	0.00
Club segment 3	0.00	20.00	0.00	8.00	0.00
Club segment 4	0.00	20.00	0.00	8.00	0.00
Club segment 5	0.00	25.00	0.00	7.00	0.00
Club segment 6	0.00	60.00	0.00	9.00	0.00
Club segment 7	1050.00	12.00	1025.00	0.00	26.00
Antenna (Total)	1050.00	149.00	1025.00	44.00	26.00

SC1: Sensillum chaetica type 1; SC2: Sesnsillum Chaetica type 2; SC3: Senisllum chaetica type 3; ST1: Sensillum trichoidea, type 1; ST2: sensillum trichoidea type 2

Table 3. Measurements of sensillum types

Sensillum type	Length (µm)		Paired t test (One tail)
	Female	**Male**	
SC1	36.47	35.296	NS
SC2	51.19	55.920	NS
SC3	32.190	29.011	NS
ST1	112.651	101.505	NS
ST2	97.184	85.920	NS

NS: Not significant

SC1: Sensillum chaetica type 1; SC2: Sesnsillum Chaetica type 2; SC3: Senisllum chaetica type 3 ST1: Sensillum trichoidea, type 1; ST2: sensillum trichoidea type 2

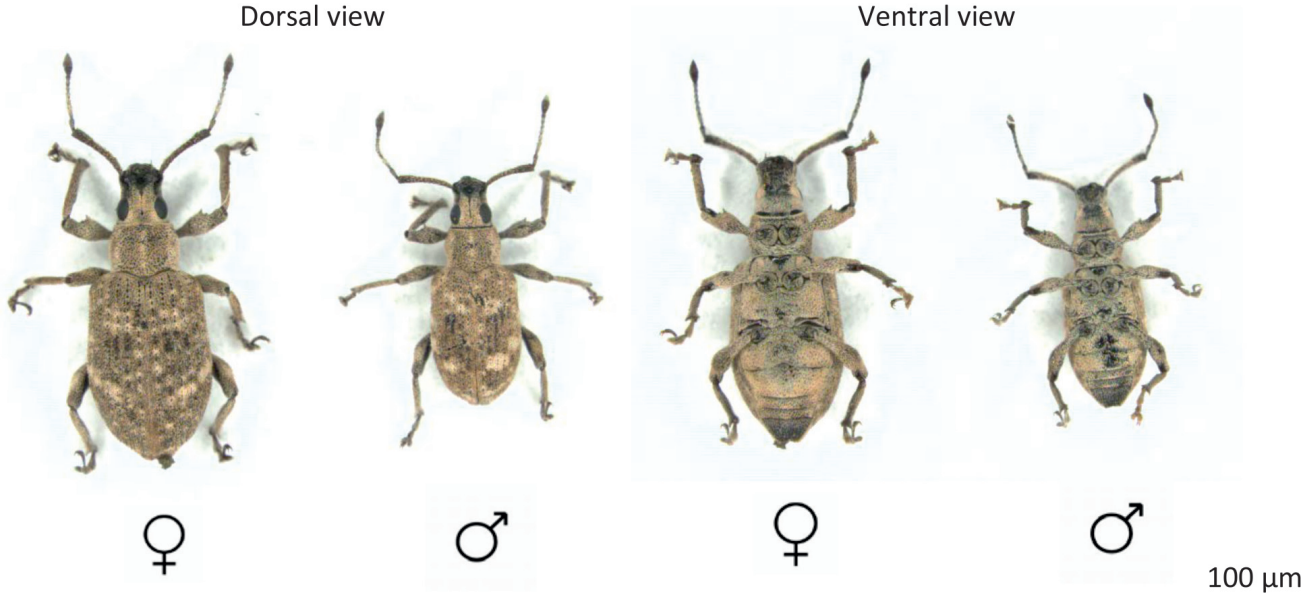

Fig 1. Dorsal and ventral view of adult male and females of *Myllocerus subfasciatus*dorsal showing size difference

Sensilla Trichodea (ST)

Among the antennal sensilla few sensilla trichodea (70) have been counted. Based on their external morphology sensilla trichodea is further divided into two subtypes Viz., Sensillum trichodea long blunt tips (ST1) and Sensillum trichodea long sharp tips (ST2).

ST1: Sensillum trichodea long blunt tips

These sensilla are long with blunt tips. These are sparsely distributed on flagellomeres 1-6, which are standing divergent to antennal surface. These are present in very few numbers (44) on flagellomere 1-6. The length of the ST1 is 1.778 mm (Tables 1 and &2).

ST2: Sensillum trichodea long sharp tips

The elongated sharp tip trichodea sensilla are mostly presently on the flagellomere 7, rest of the flagellomeres are devoid of the sharp tipped trichodea sensilla.These sensilla are found in a very low numbers(26.00) compared to the subtype blunt tip trichodea (Table 2). The length of the ST2 is1.601mm (Tables1 and 2).

The length of the different sensilla *viz.,* SC1. SC2, SC3, ST1, ST2 in female and male weevils are statistically not significant (Table 3). The length of the scape is statistically significant in female (1507.72 µm) and male (1394.62 µm) weevils (p=0.008). Whereas, there was no significant difference observed in the length of the funicle and club segments. The length of the funicle in female (308.533 µm) and male (299.621 µm). The length of the club segments 1-7 in females ranged from 123.07 -637.14 µm and in males ranged between 124.942-635.688 µm (Table 4).

Sexual dimorphism

Morphologically the male and female ash weevil *M. subfasciatus,* looks similar with respect to colour, shape, except the size. The adult female is bigger in size in comparison to males (Fig. 1). The average mean weight of the females (*P<0.0001;t=7.445;df=9*) are significantly more than males. The average weight of the female individual is 49 ± 4.81 mg ranging from 20-70 mg, whereas, males 12.1± 0.50 mg ranging form 10-14 mg.

The body length of adult female ranged from 4-5mm and male adult weevil is 3-3.2mm. The mean body length and width of the female (4.58 ± 0.013; 1.75± 0.00) is also significantly larger in comparison with the males (3.16 ±0.01; 1.05±0.00)respectively (*P<0.001; t=74.85; df=9*).Significant difference observed in length and width of thorax in males and females (*P<0.0001; t=7.718; df=9*) (*P<0.0001; t=11.46; df=9*) respectively. The length of the abdomen was significantly larger in females (3.05 ± 0.01) than males(2.35 ±0.01) (*P<0.0001; t= 21.96;df=9*). Similarly, the width of the abdomen was also significantly greater in females than males (*P<0.0001; t=31.42; df=9*). (Tables 5 and Fig. 8).

The males have more pronounced creamy white-coloured markings on the elytra compared to females. The females have less creamy markings on the elytra region. Presence of single spine like structure was observed on prothoracic femora of both the sexes of *M. subfasciatus*.

Table 4. Measurements of different antennomeres in female and male *M. subfasciatus*

Antennomere	Female		Male	
	Length (µm)	Circumference (µm)	Length (µm)	Circumference (µm)
Scape	1507.72	0.009	1394.62	0.008
Funicle	308.533	0.037	299.621	0.032
Club segment 1	167.227	0.064	166.126	0.062
Club segment 2	150.006	0.070	137.981	0.074
Club segment 3	156.554	0.077	163.264	0.063
Club segment 4	125.076	0.086	124.941	0.082
Club segment 5	136.047	0.060	153.501	0.067
Club segment 6	157.927	0.061	158.226	0.065
Club segment 7	637.149	0.013	635.688	0.014

Table 5. Morphological parameters of male and female ash weevil, *M. subfasciatus* (Mean and range n=10)

	Male				Female				
	Length (mm)	Range (mm)	width (mm)	Range (mm)	Length (mm)	Range (mm)	width (mm)	Range (mm)	P value
Body	3.161 ± 0.011	3.098 - 3.204	1.056 ± 0.00	1.02 - 1.08	4.586 ± 0.013	4.529 - 4.664	1.75 ± 0.00	1.67 - 1.78	<0.000
Head	0.603 ± 0.00	0.54 - 0.661			0.73 ± 0.00	0.698 - 0.756			<0.000
Thorax	0.603 ± 0.00	0.601 - 0.611	0.887 ± 0.00	0.877 ± 0.898	0.654 ± 0.00	1.00 -1.089	1.054 ± 0.012	0.623 -0.666	<0.000
Abdomen	2.35 ± 0.019	2.23 - 2.401	1.056 ± 0.00	1.02 - 1.08	3.05 ± 0.016	3.016 - 3.123	1.75 ± 0.00	1.67 - 1.78	<0.000
Antennae	1.04 ± 0.00	1.03 - 1.04	-	-	1.75 ± 0.00	1.73 - 1.79	-		<0.000
Weight (mg)	12.1± 0.50 mg	10-14 mg	-	-	49 ± 4.81 mg	20-70 mg	-	-	

The last abdominal sternites of males found more white accumulation presumably cuticular secretions (Fig. 4A). The presence of secretory pores observed in the last abdominal sternite in both male and female adults (Fig. 4B and E). These secretory openings are responsible for secreting the cuticular secretions. In SEM images structural difference in the cuticular secretions were observed in males and females. The females cuticular secretions are small parallel tubular granule like structures which are in multiples, whereas male cuticular structures are clustered irregular shaped structures (Fig. 7A). Large quantities of cuticular secretions were found on male abdominal sternite compared to minimum cuticular secretions on the female abdominal sternite (Fig. 7B).

Morphologically the thoracic region of both male and female are the same. But when we dissected out the thoracic part observed slight difference in the structure. The thoracic glands are balloon like structures with an extension towards the abdomen (Fig. 5 A-C). Upper portion of thoracic gland of female is bulged and narrow tube-like structure. Where as in males the upper part of the thoracic glands wasobserved with presence of small openings. These opening may be responsible for releasing some compounds which are responsible for communication (Fig. 5 D-F). The chemical ecology experiments in this line are in progress.

Body scales

The adult weevils are long and broad with convex dorsal surface (More pronounced in females) and flat in ventral surface. The adults have habiliment of different types of scales distributed all over the body (Fig. 6). The female body is sparsely distributed with ovate and elongated scales. Whereas, males are densely covered with ovate and elongated scales (Fig. 6). The SEM images of elongated and ovate scales are seen with the cuticular secretions.

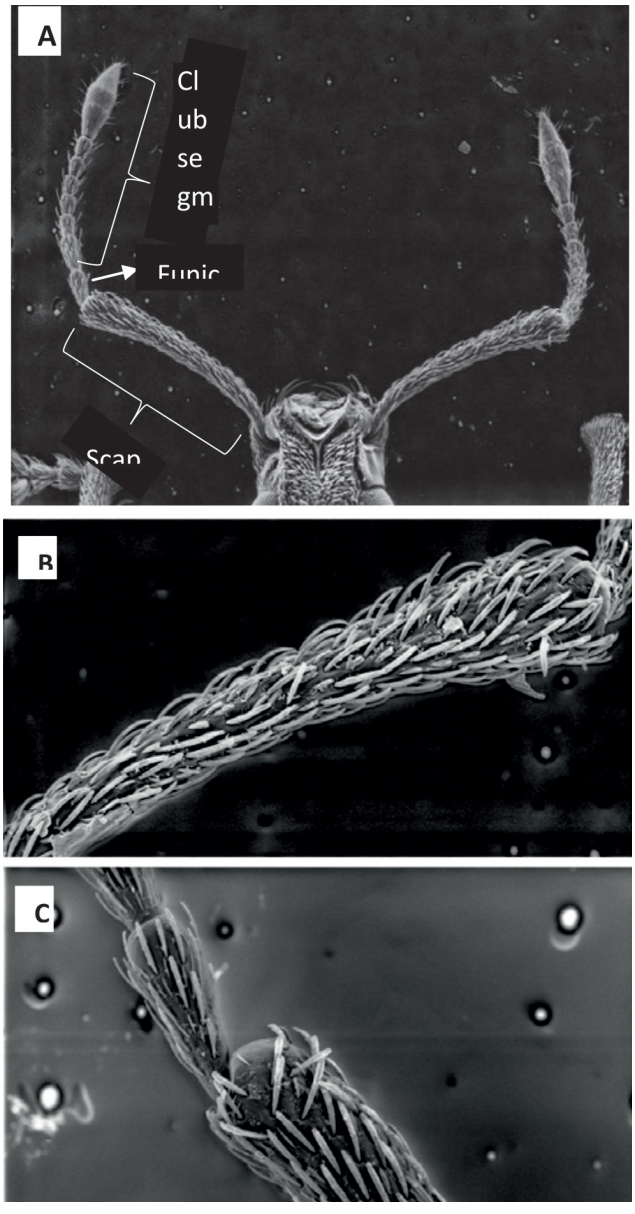

Fig 2. Geniculate type of antenna. SEM image of Scape and pedicle region of antenna, with elongated scales devoid of sensilla trichoidea.

The SEM image revealed two different types of scales on the head, antennae, thorax, abdomen regions viz., ovate and elongated scales with ridges surfaces. The length of the elongated scales are slightly bigger in females compared to males. These scales were observed with cuticular secretions distributed all over the body.

Reproductive systems

Attempts were made to dissect the male and female reproductive organs of *M. subfasciatus*. The female reproductive system contains a pair of ovaries, a spermatheca and ovipositor. The male reproductive system contains a pair of testis, aedeagus, seminal vesicle and ejaculatory duct. Morphologically the adults

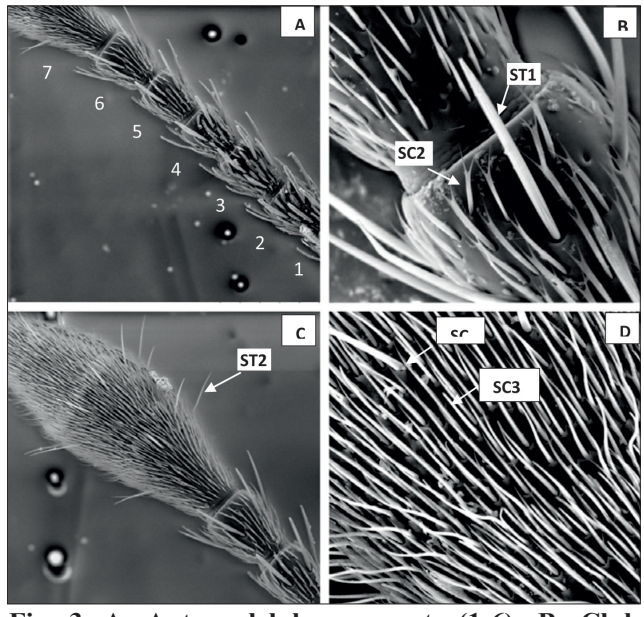

Fig. 3. A: Antennalclub segements (1-6); B: Club segments 4 and 5 with forked shape sensilla SC2 & ST1; C: Last flagellomere dome shaped dense trichoidea sensilla (ST2); D: Sensilla Chaetica at higher magnification

are separated based on the size and weight. The female adults are longer, bigger, and heavier in comparison with the males. Denser deposition of white cuticular secretions occurred in males than in females. The structure of these cuticular secretions varies with males to females. In females, presence of irregular large cuticular secretions observed, but in males small parallel granular secretions are being observed in SEM image analysis.

Field collected females of *M. subfasciatus* weevils are longer, broader and heavier than males. This significant differences in size makes initial separation of sexes easier when both sexes are available for comparison. There was no significant difference observed in the antennal segments and sensilla of male and female weevils except length of the antenna. Denser cuticular secretions at last abdominal sternites were observed in male weevils as compared to the female weevils. Significant difference in the thoracic glands was observed in males with small openings in the upper part, but absent in the female thoracic glands. This disparity will further confirm the sexes of ash weevil *M. subfasciatus* for researchers.

ACKNOWLEDGEMENTS

We are thankful to the Director, ICAR-IIHR, Bengaluru for providing facilities to conduct the work. My sincere thanks to Dr. M. Krishna Reddy, Head, Division of Crop Protection, ICAR-IIHR, Bengalurufor all his support and Dr. Samuel, Principal Scientist, Division of Crop Protection, ICAR-IIHR, Bengaluru

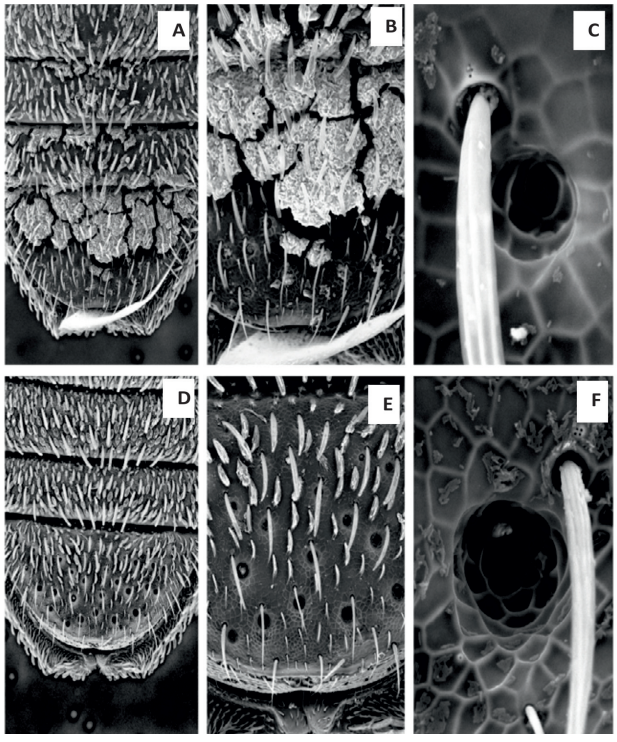

Fig 4. SEM images of abdominal sternites A, B: Ventral view of abdominal sternites with cuticular secretions and openings A,B: in male; D,E: in female; Cuticular openings on sternite with the scaleC: on male weevil; F: on female weevil

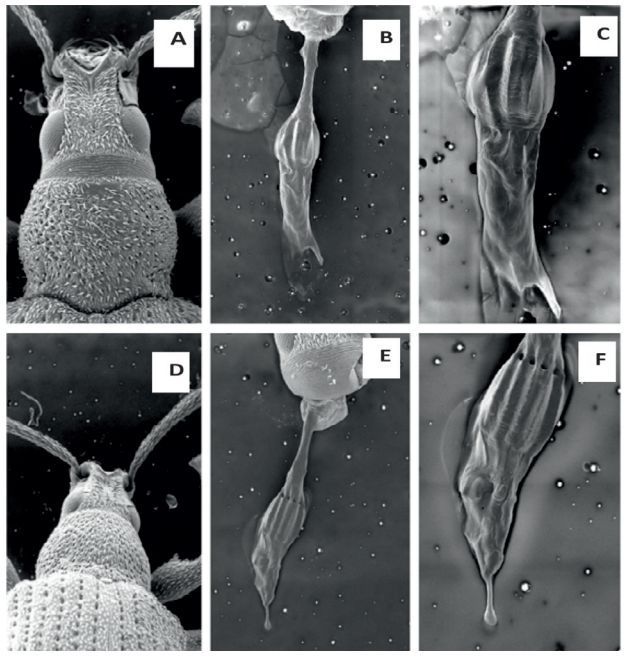

Fig 5. A, D: Thoracic region of male and female *M. subfasciatus;* B,C: Thoracic glands of female, Balloon like structure without openings ; E,F: Thoracic glands of male adult with circular openings

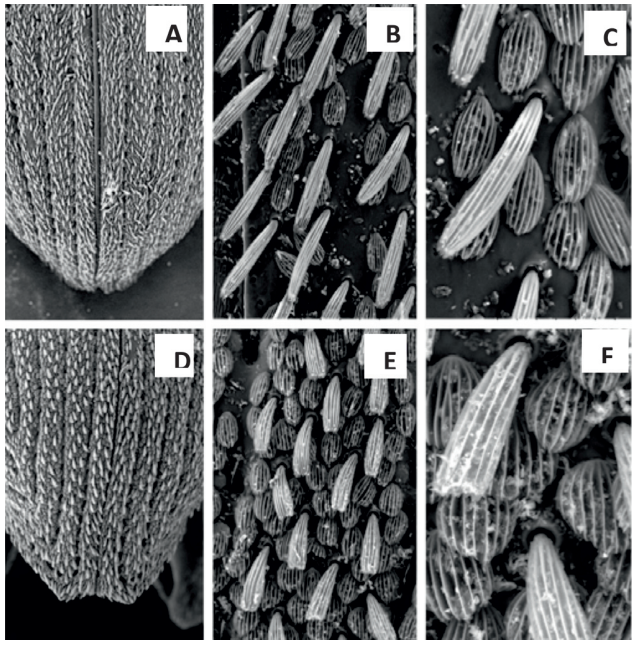

Fig 6. Habiliment of different types of scale on Female (A) and Male (D) body; B & C: SEM images of ovate and elongated scales of female distributed sparsely. E& F: SEM images of scale distributed densely on male body.

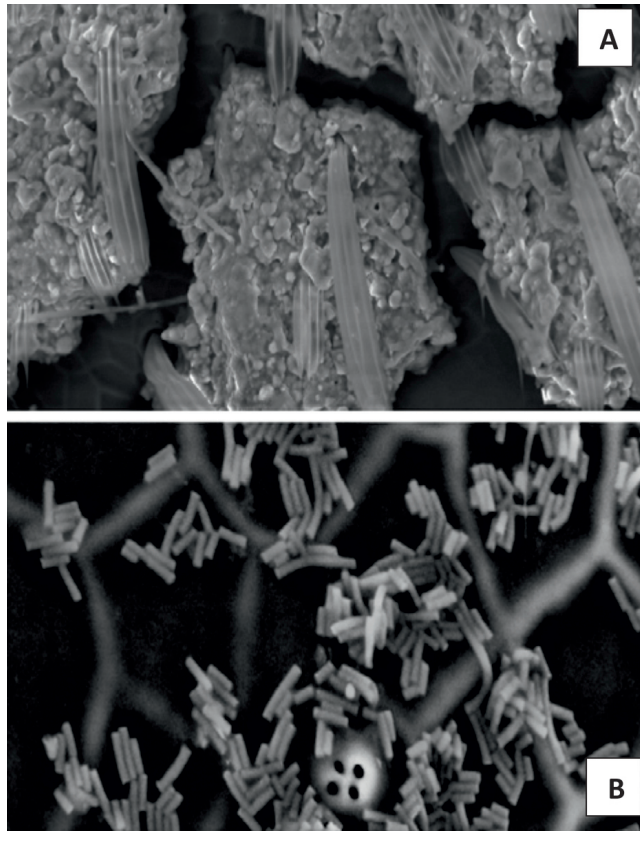

Fig 7. SEM images of Scales and Cuticular secretions of Male weevil (A) and Female weevil (B)

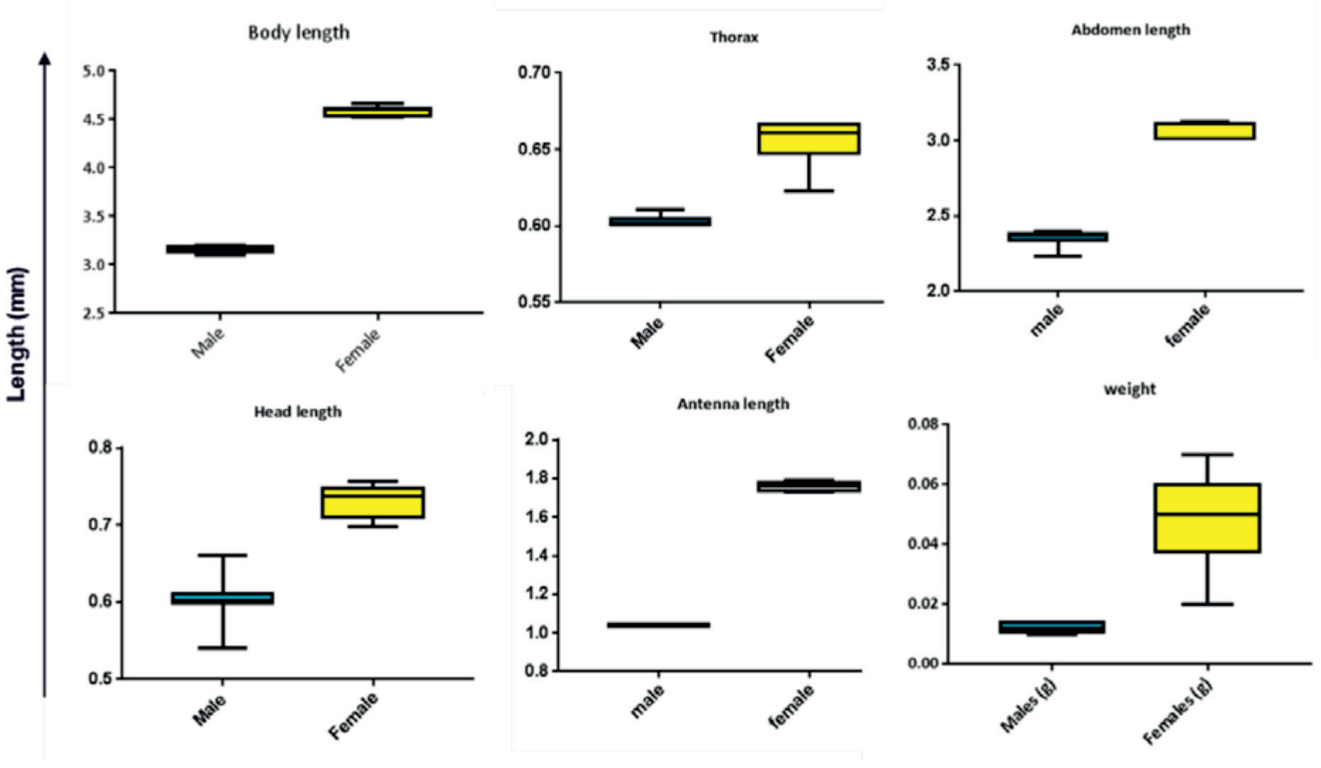

Fig. 8. Morphological measurements of male and female ash weevil, *M. subfasciatus*

for providing the SEM facilities. Authors thank Dr. K. Sreedevi, Principal Scientist, ICAR-NBAIR, Bengaluru, for her support.

REFERENCES

Nair, M. R. G. K. *Insects and mites of crop pests in India*. Indian Council of Agricultural Research, New Delhi, 1986

Padmaja P.G. 2016. Biotic Stress Resistance in Millets, Indian Council of Agricultural Research, New Delhi, 2016

Ramamurthy, V. V. and S. Ghai. 1988. A study on the genus *Myllocerus* (Coleoptera: Curculionidae). *Orienal Insects*, **22**: 377-500.

Shanmugam, P. S., Srinivasan, T., Baskaran, V. and Satiah T. 2021. Ash weevil *Myllocerus subfasciatus* Guerin-Meneville (Coleoptera; Curculionidae) – An emerging threat to brinjal cultivation. *Biotica Research Today*, **3** (5): 376-378.

MS Received 15 October 2021
MS Accepted 26 November 2021