2024 SEM Ultrastructure Study of *Lygus hesperus* (Knight) (Hemiptera: Miridae)

Mr. Ram B. Shrestha , Texas Agricultural Experiment Station, Lubbock, TX
Dr. Megha N. Parajulee , Texas Agricultural Experiment Station, Lubbock, TX
Dr. Mark J. Grimson , Texas Tech University, Lubbock, TX

ABBREVIATIONS: SEM (Scanning Electron Microscopy)

Abstract

Lygus hesperus (Knight) is an economically important insect pest in the United States. This genus is comprised of 43 species worldwide (Kelton, 1975), out of which 34 species are recorded from the United States. Identification of these species using macro-structure morphology and taxonomic key is very difficult. With advancement of Scanning Electron Microscopy (SEM) it is possible to investigate ultra structures of any organism therefore has been commonly used in systematics of various organisms. The objective of this study was to evaluate the SEM ultra structure of *Lygus hesperus* and compare with published data on other species. The ultra structures of adult *Lygus hesperus* were studied under scanning electron microscopy. Five adult live *Lygus hesperus* were fixed with half strength Karnovsky's fixative and chemically dehydrated with graded series of alcohol. SEM micrograph was obtained after critical point drying and sputters coating with gold/pladdium. Fifty different body parts were measured and details on various structures have been discussed. Eggs of *Lygus hesperus* were found smaller than that of *Lygus lineolaris*. The pronotal sensilla were the longest among the various sensilla found on its body. SEM ultra structure study is one of the potential methods to differentiate *Lygus* species.

Key words: *Lygus hesperus,* Morphometry, Scanning electron micrograph, Ultra structure.

Introduction

Lygus species (Hemiptera: Miridae) is an economically and ecologically important group of insects in row-crop agroecosystems. This genus is comprised of 43 species worldwide (Kelton, 1975), out of which 34 species are recorded from the United States. The genus *Lygus* was first described by C. W. Hahn (1833). This genus once contained many species (about 300) but with a series of revisions and refinements, using some additional characteristics such as genitalia and electron microscopy, many previous subgenera of *Lygus* were elevated to generic status (Thomas and Lattin, 1987). Significant revisions of this genus were done by Knight (1917), China (1941), Slater (1950), Leston (1952), Kelton (1955), Wagner (1957), and Carvalho et al. (1961). Thomas and Lattin (1987) also critically reviewed the taxonomic status of the genus *Lygus* and concluded that the *Lygus* complex exhibits greater morphological variation and is therefore very difficult to positively identify. Accurate identification of *Lygus* species in the nymphal stage is not possible. During a *Lygus* survey in Texas, we also faced similar difficulties in positively identifying some individuals because of the limited taxonomic keys to females and the unexpected temporal variation in adult *Lygus* collected from different hosts. This difficulty in positive identification to species hinders the development of species-specific management programs (Thomas and Lattin, 1987).

Classical taxonomic keys are written by taxonomists for taxonomists' use and are not easy to use by crop managers. Identifying the species by the current taxonomic key requires extensive taxonomic expertise. Therefore, the need for some user-friendly methods of identification is urgently apparent. We speculated that morphometric quantitative characteristics (color, shape, size and texture) of different body parts with multivariate data analysis will enable us to delineate the species and result in more accurate identification than qualitative keys. However, with traditional macro-morphometry, species level identification was not clear. There is always a continuum of overlapping variables that makes the species discrimination by macro-morphometry difficult.

Morphometric study of *Lygus* using light microscopy and digital image analysis has been initiated in our laboratory. In such study only the larger, visible body parts of *Lygus* are studied under stereo microscope and images acquired are only up to 40 times magnified. But this method could not clearly distinguish the species thus the need for an ultra structure study with higher resolution scanning electron microscope. Systematists are constantly searching for better characters in classifying the organisms. With the advancement of SEM it is possible to investigate ultra structures of any organism. SEM ultra structures have been commonly used in systematics of various organisms like fungi (Cole, 1979), protozoan, nematodes, plants (Tyler, 1979; Stuessy, 1979; Pickett-Heaps, 1979), angiosperms and other invertebrates (Storch, 1979), but it has been rarely used in insect systematics. Chinta et al. (1997) studied antennal sensilla of *Lygus lineolaris* while Hatfield and Frazier (1980) reported on labial tip sensilla and Ma and Ramaswamy (1987) studied ovarian histology. Ma et al. (2002) studied egg morphology of *Lygus lineolaris* using SEM. Recently Romani et al. (2005) studied the mouthparts of *Lygus rugulipennis* using Transmission Electron Microscope. However, there is no report on *Lygus hesperus* on ultra structure study. This study was designed to investigate the possibility of application of various ultra structure characteristics of *Lygus* adult in classification of *Lygus* complex. The objective of this study was to evaluate the SEM ultra structure of *Lygus hesperus* and compare with published data on other species. Specifically, we aimed to standardize the sample preparation and imaging technique for *Lygus hesperus* scanning electron microscopy that will be applied in future in detail ultra structure phylogeny of *Lygus*.

Materials and Methods

Adult *Lygus* specimens were collected from an alfalfa field in Lubbock County, Texas, using sweep-net sampling and separated individually and then killed by freezing in 1.5 ml micro centrifuge tubes at -20 ^{o}C. Out of 100 specimens collected, 5 male and 5 female adults were selected randomly for SEM analysis while the remaining samples have been saved as voucher specimens in the Cotton Entomology Program laboratory in Lubbock.

Specimens for SEM were prepared following Hatfield and Frazier (1980). Antennae, front leg and genitalia of adult *Lygus* were fixed in half-strength Karnovsky's (1965) fixative overnight, rinsed 3 times in phosphate buffer (pH 7. l), post-fixed in 2% OSO at 4 °C for 4 h, and rinsed 3 times in distilled water and dehydrated in a graded series of ethanol (series of 10, 30, 40, 60, 70, 90, 100, 100% ethanol for 30 minutes in each concentration). Specimens were then air-dried or critical point-dried and mounted on aluminum stubs using carbon tape and aluminum paint. They were coated with gold/palladium and examined with following SEM settings: accelerating voltage= 15 kv, current= 60 uA, working distance= 30, 15, 10 variable, aperture= number 3, tilt= no tilt, condenser= both 1^0 and 2^0 at 5, detector=lower detector and stage=lower stage.

The SEM digital images were saved directly in PC and resized using Irfanview software. Fifty different body parts were measured using digital image analysis Motic Image Advance 3.2

Software. The specimens will be saved in laboratory for future reference. For each body part, images from five different insects were measured and average data are reported.

Results and Discussions

SEM micrographs were taken for different body parts of 5 adult *Lygus hesperus* and fifty different body parts were measured from the SEM micrographs. The detail measurements have been presented in Figures 1-8 and in Table.1. All the measurements were in micro meter (μm). All the positions of measurements have been shown by dotted lines in Figures 1-8 and the different body parts within a figure has been labeled with serial number.

Lygus hesperus has 2.13 mm wide pronotum 1.14 mm wide scutellum and 2.09 mm long rostrum (fig. 1). Whole body was covered with fine sensilli. Pronotal sensilla were the longest (80 μm) followed by tarsal peg sensilla (73.4 μm), cuneal sensilla (71.1 μm), first antennal segment sensilla (55 μm), scutellum sensilla (50 μm), fourth antennal segment sensilla (42.7 μm) and tarsal small sensilla (37.5 μm). These sensilla are the organs for sensing various chemical stimulus and physical vibrations as well as to protect the body surface from physical factors. In this study, various types of sensilla were not identified rather they were grouped based on their place of origin. In future, further categorization of these sensilla along with their distribution patterns and density will be determined.

Lygus hesperus eggs were cylindrical (950 μm length and 198-267 μm width) and has flat elliptical operculum with fine opening for aeration of the growing embryo. The average length, width, and circumference of elliptical operculum were 216, 110, and 503 μm, respectively (figs. 5 and 6). Eggs of *L. hesperus* were slightly smaller than that of *L. lineolaris* which is in agreement with Ma et al. (2002). Front coxa, trochanter, femura, tibia and tarsus were 563, 183, 1023, 1129 and 482 μm long, respectively. The anterior end of the tarsus was furnished with a pair of claw (115 μm long) and fan like arolium (48.9 μm long). The female genital opening was 1.1 mm long. It has a long ovipositor made up of a pair of primary valvule and another pair of secondary valvule. The length of valvule was 1.12 mm which helps in creating holes in soft plant tissues and injecting the eggs inside.

This study showed that the critical point drying and use of fresh (live) sample is very critical for better imaging. Use of alcohol stored samples had problems of shrinkage so the measurement could not be done accurately. The quality of SEM micrograph was similar with or without the use of secondary fixative (2% OSO_4). Therefore, we suggest that the secondary fixation with OSO_4 is not necessary. Sputter coating for about 1 min 30 seconds is needed and imaging will be better in 15 kv accelerating current. Higher voltage such as 20-25 kv will damage the sample in higher magnification.

Conclusions

Ultra structure study of *Lygus hesperus* using SEM gives greater resolution and depth of focus thus better results as compared to the light microscopy. Even though the process of imaging in SEM is time consuming and required sophisticated SEM, critical point dryer and sputter coating equipment, it is worthwhile to apply this technique for precise and detail mophometry if these equipment are available in the laboratory.

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Table 1. Different body parts measured by digital image analysis of scanning electron micrograph of *Lygus hesperus.*

SN	Figure #	Part #	Name of body part	Size (µm)		SN	Figure #	Part #	Name of body part	Size (µm)
1	1a	1	Pronotum width	2137.8		26	4b	2	Length of claw	115.9
2	1a	2	Scutellum width	1146.2		27	4b	3	Length of arolium	48.9
3	1a	3	Clavus width	371.1		28	4b	4	Tarsal sensilla length	37.5
4	1a	4	Pronotal sensilla length	80.0		29	4b	5	Tarsal sensiella base width	2.8
5	1b	1	Rostrum length	2093.4		30	5a	1	Tibial peg sensilla length	73.4
6	1b	2	Front coxa length	563.9		31	5a	2	Tibial Peg sensiella width	11.9
7	1b	3	Trocanture length	183.5		32	5a	3	Small tibial sensilla length	44.6
8	1b	4	Femura length	1023.1		33	5a	4	Small tibial sensilla width	3.6
9	1b	5	Tibia length	1129.4		34	5b	1	Peg sensilla width	10.0
10	1b	6	Tarsus length	482.8		35	5b	2	Groove width in peg sensiella	1.0
11	2a	1	Distance between antennae	537.3		36	6a	1	Length of scutellum	757
12	2a	2	Labrum length	633.6		37	6a	2	Side length of scutllum	783
13	2a	3	labrum width	191.9		38	6a	3	Scutellum sensilla length	50
14	2a	4	Front width	386.5		39	6b	1	Cuneous notch	438.8
15	2b	1	Male genitalia elliptical width	576.5		40	6b	2	Cuneous external curvature length	1004.6
16	3a	1	Egg length	950.9		41	6b	3	Cuneal sensilla length	71.1
17	3a	2	Egg width at neck area	198.8		42	7a	1	First antennal segment length	445.7
18	3a	3	Egg width at bottom area	267.0		43	7a	2	First antennal segment width	91.3
19	3b	1	Operculum length	216.2		44	7a	3	First antennal Sensilla length	55.0
20	3b	2	Operculum width	110.0		45	7b	1	Fourth segment of antennae width	82.4
21	3b	3	Operculum circumference	503.5		46	7b	2	Sensilla length of 4th antennae segment	42.7
22	4a	1	Posterior segment of tarsus	181.8		47	8a	1	Female genital opening length	1129.6
23	4a	2	Middle segment of hind tarsus	177.1		48	8b	1	Valvule length	1118.3
24	4a	3	Anterior segment of hind tarsus	223.7		49	8b	2	Valvule width at posterior head part	288.9
25	4b	1	Thickness of hind tarsus	56.0		50	8b	3	Valvule width at anterior part	174.5

Note: All measurements were average of 5 specimens and all body parts were from female specimens, except for male genitalia.

Figure 1. a) Dorsal view of anterior body of *Lygus hesperus* (head and thorax); b) Ventral view of anterior body (head and thorax). Refer this and following figures to Table 1 for description of body parts.

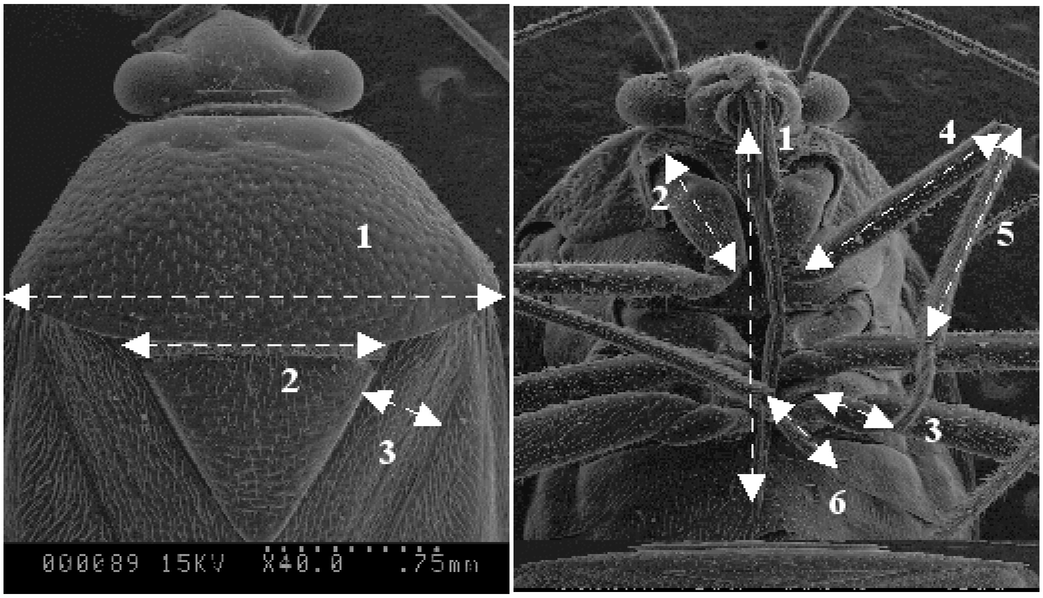

Fig. 1

Figure 2. a) Frontal view of head; b) Rear view of posterior genitalia of male specimen.

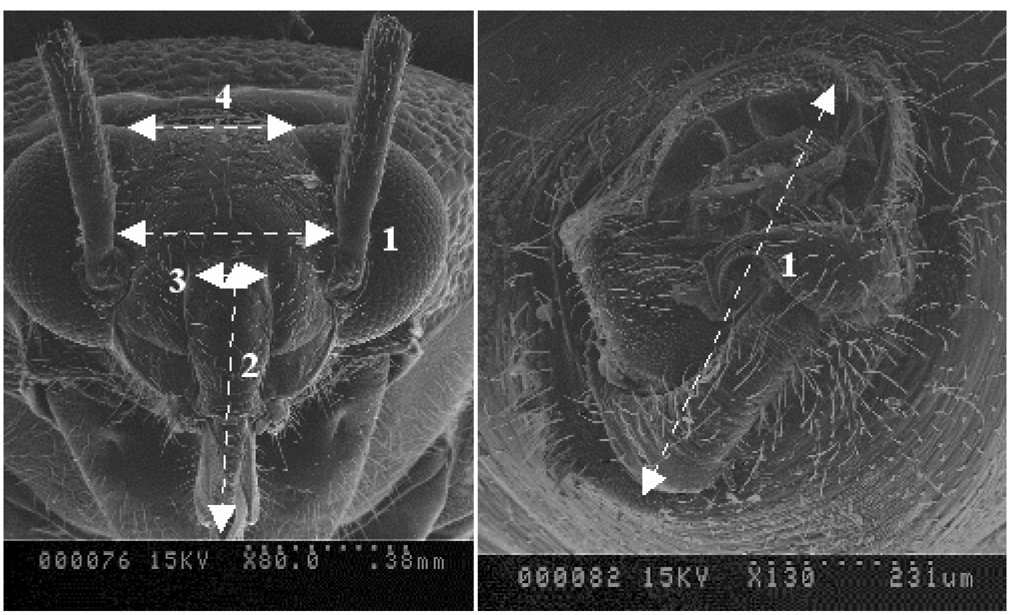

Fig. 2

Figure 3. a) Egg; b) Operculum of the egg.

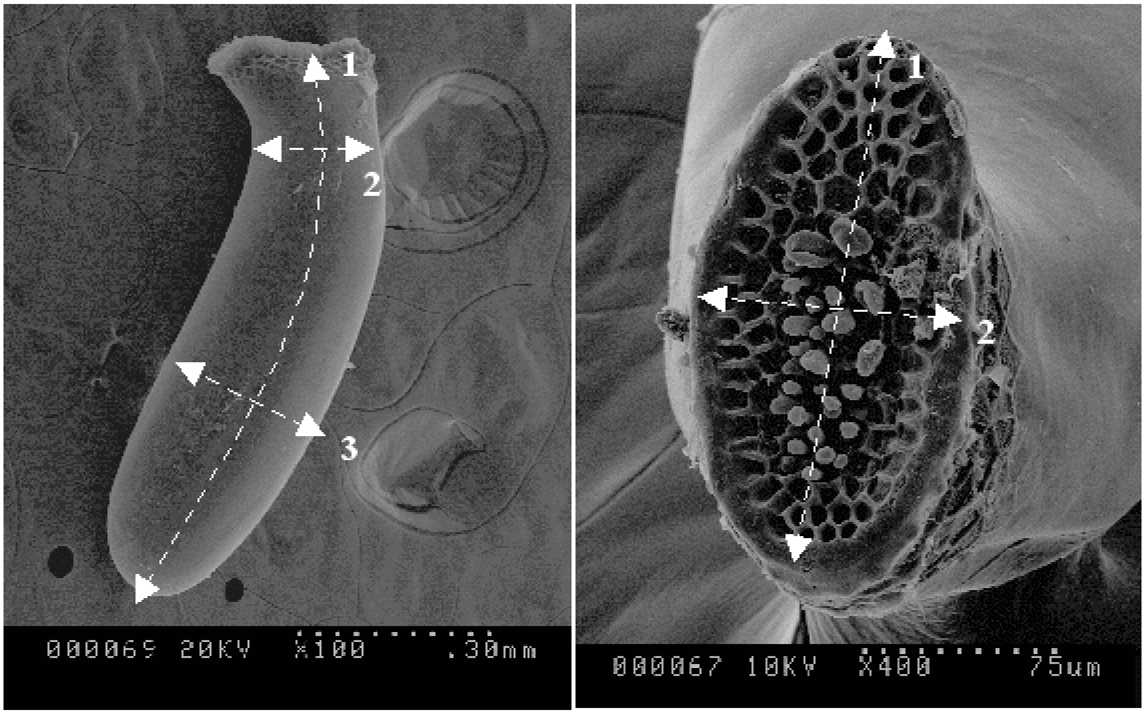

Fig. 3

Figure 4.a) Tarsus; b) Claw.

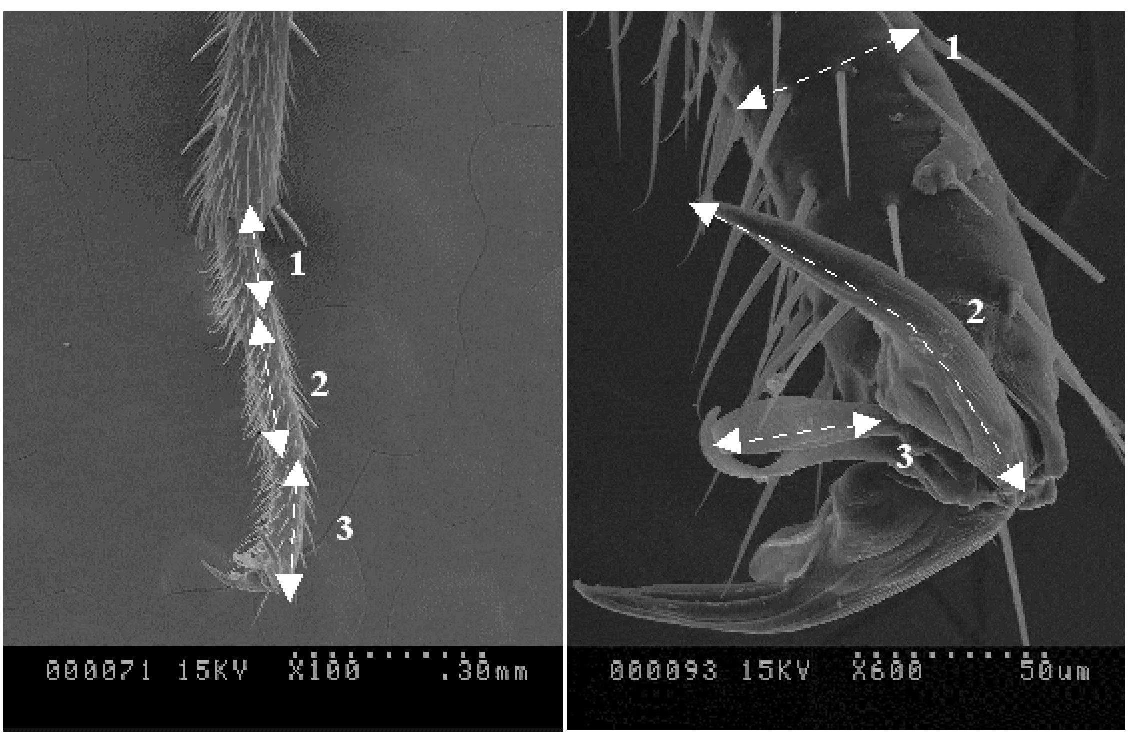

Fig. 4

Figure 5. a) Tibia and tarsus joint; b) Peg sensilla grooves.

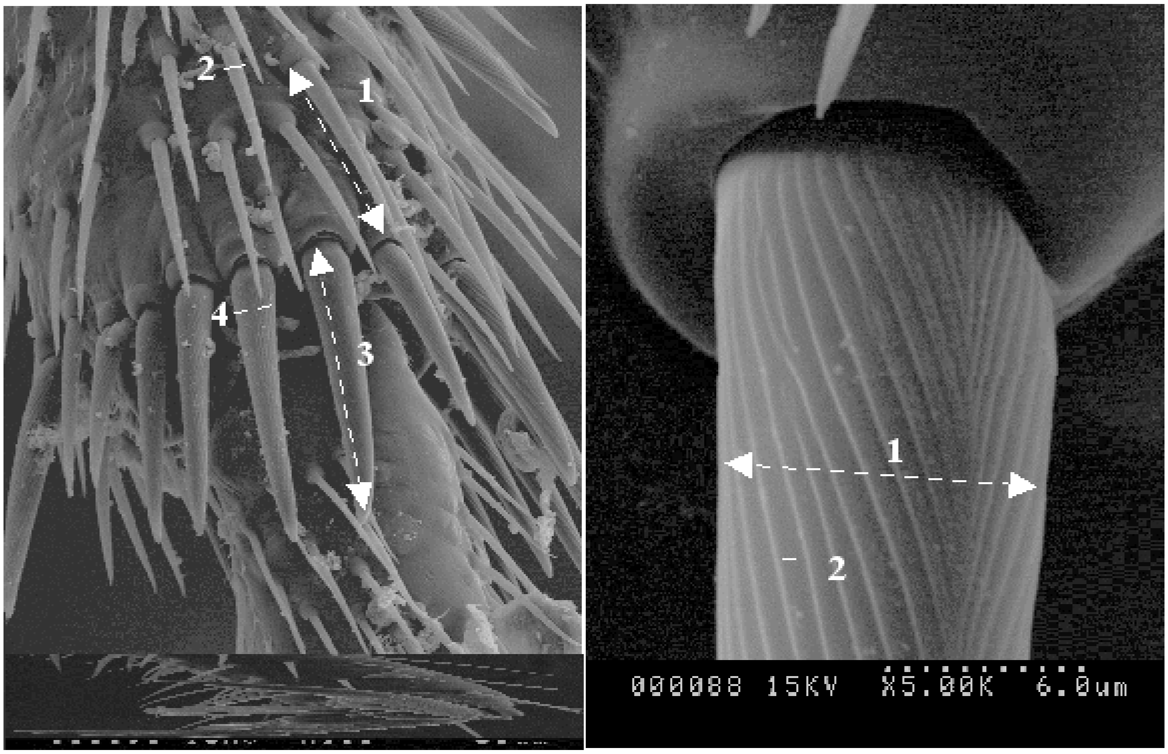

Fig. 5

Figure 6. a) Scutellum; b) Cuneus.

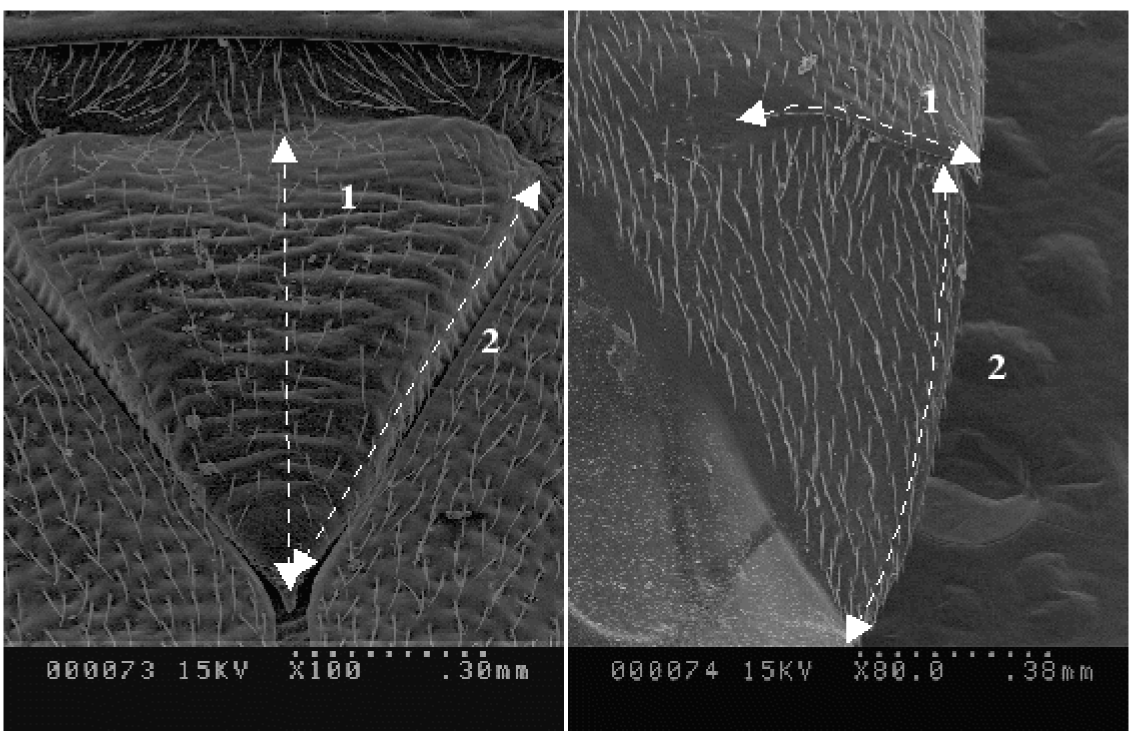

Fig. 6

Figure 7. a) First segment of antennae; b) Fourth segment of antennae.

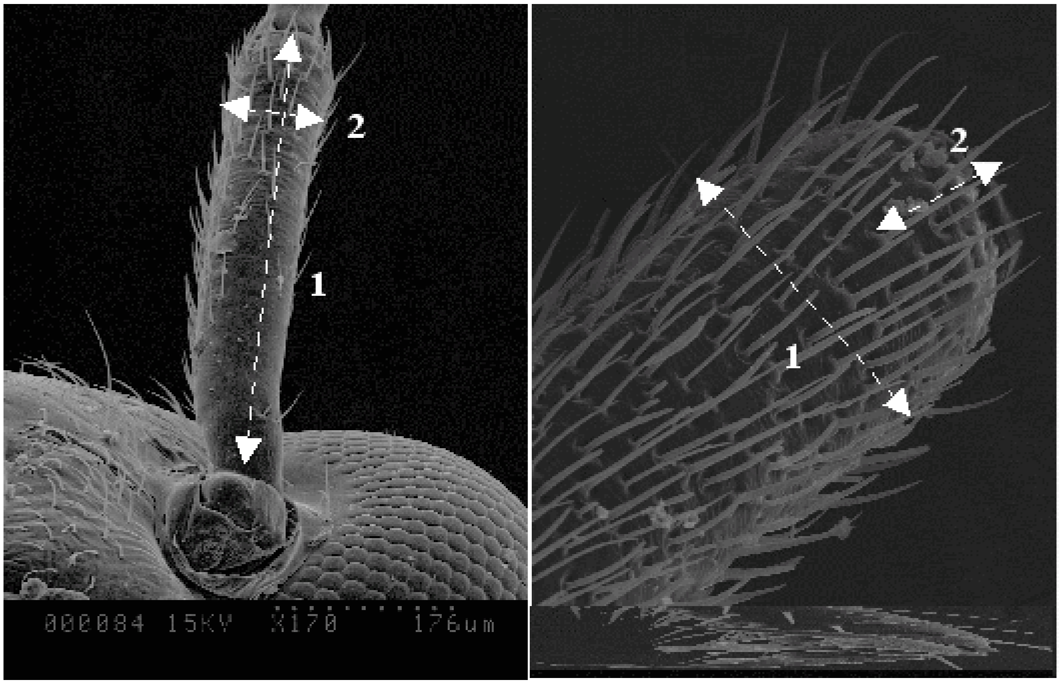

Fig. 7

Figure 8. a) Ventral view of abdomen; b) Ovipositor (Valvule).

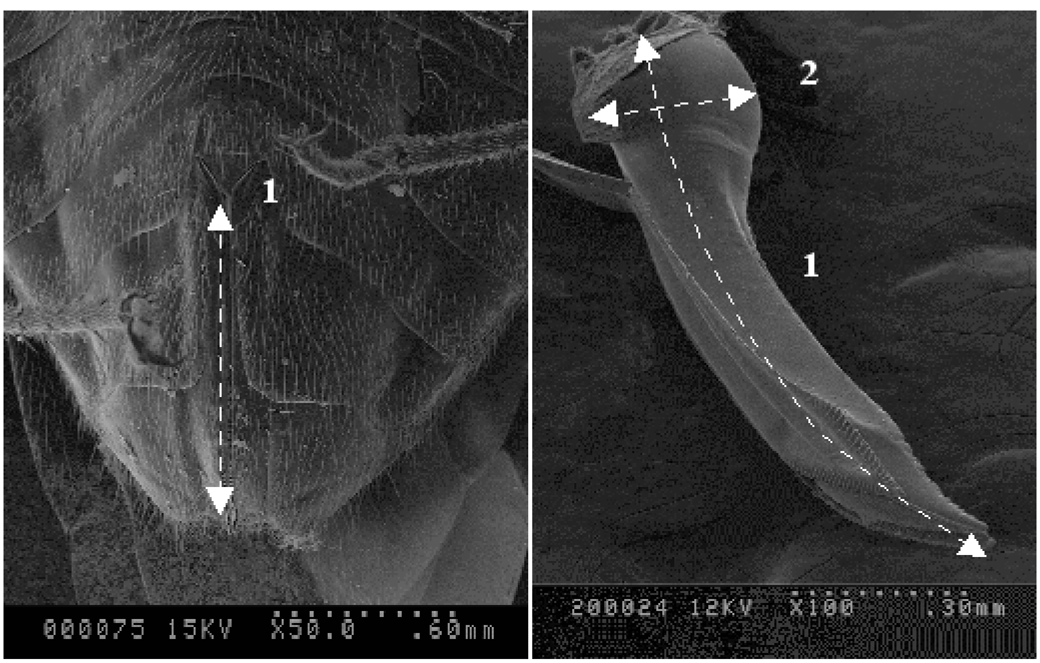

Fig. 8