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Identification Studies on Chrysomya Bezziana (Diptera: Oestridae) Larvae Infested Sheep in Eastern Region, Saudi Arabia

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Abstract: ***Ultrastructure study revealed (Villeneuve, 1914) Chrysomya bezziana that causes myasis in sheep in Saudi Arabia, the studies recorded the exact composition of genus showed differences in morphological characteristics, which cannot be identified using a microscope optical as well in all previous studies the same region as never before studied.***

Keywords: Myasis, SEM, Saudi Arabia, sheep, Chrysomya bezzian

1. Introduction

Researches have recorded the presence of myiasis in some studies conducted in Saudi Arabia in territories of camels and sheep breeding. Also, cases of myiasis were recorded in the slaughterhouses of Jeddah, Riyadh and the eastern region, where the larvae were found attached to the mucous membrane of the nasal passages, sinuses and throat, causing severe irritation and changes in tissues. The larvae sometimes reach the cranial cavity, causing neurological disturbances that may lead to death. Larvae also scratch the mucous membranes, which leads to the entry of various microbes which causes death **(Husseinet. al, 1981**). Moreover, these larvae were used to clean wounds as they feed on living tissues. They are significant because they cause myiasis to the livestock, infecting animals of economic value, such as sheep and cattle, and sometimes humans.

Chrysomya bezziana, also known as Screwworm Fly, is a parasite that infects mammals. The larvae of this parasitic flies need a host to complete their growth. Those larvae are widely spread throughout the world, in Asia, Middle East, Indonesia, Philippines, tropical Africa, India and Guinea; however, there is no evidence for their existence at altitudes higher than 2500 meters above sea level **(Sutherst et al, 1998)**. In some areas, no detection was reported for such larvae. The adult fly has a green or blue color and has a golden head, while larvae are smooth and feed on the tissues of living and dead mammals. These larvae cause lesions to living animals.

The adult fly feeds on decaying organic matter, while the fly's larvae feed on the living tissues of warm-blooded mammals instead of the dead tissues on which many of the fly's larvae feed. As larvae can cause permanent tissue lesions, *C. bezziana* has received a lot of attention because it is considered a festering wound-cleaning larva since these larvae can actively shelter through living tissues. Many recent studies of *C. bezziana* were conducted for their medical importance.

The medical significance of these larvae, *C.bezziana*, is that they usually infect cattle, causing Myiasis. Myiasis is the invasion of tissues (living or dead ones) of a living mammal by the fly's larvae. It can run rampant to mammals such as sheep, dogs, cows, pigs, and even humans.

The adult female lays eggs on superficial wounds in living animals preferring wounds that are several days old **(Boonchu *et al* 2005)**. *C. bezziana* eggs are usually laid in the navel of newborn cattle species or on the castration cuts of cattle. The eggs are also laid on open sores, ulcers, and external abrasions or on the mucous membranes all over the body. Small wounds such as a tick bite are large enough for a female to lay her eggs.

Once the eggs hatch, the larvae burrow into the living animal's tissue and feed on it. Its common name, "screwworm", is derived from worms that insert themselves into the flesh of their hosts in a screw-like manner. Larvae can penetrate to a depth up to 15 cm (5.9 inches) into the host's living tissue. When the larvae feed and cause tissues decay, the wound produces a distinct odor that cannot be noticed by humans. However, this wound attracts the female and encourages her to lay her eggs there as well, causing further infection **(Boonchu *et al* 2005)**.

C. bezziana differs from other flies in that tissue infection can occur without the presence of dead tissues. *C. bezziana* worms can cause serious and permanent tissue damage. infested wounds can mostly lead to death if left untreated. Mature adults of this fly feed on decaying corpses, decaying matters, feces, and flowers. Due to their diet, these adult flies can be a mechanical carrier for pathogens **(Boonchu *et al*, 2005).**

Forensic entomologists can identify the closest time of death. Flies could have attacked dead bodies by means of adult insects instead of letting them die without the benefit of feeding on them, which does not always coincide with the time of death. The attraction of flies to dead bodies helps to identify their location and find them **(Boonchu *et al*, 2005).**

In Saudi Arabia, some studies indicate the existence of myiasis in humans **(Ansari and Oertley, 1982; Kersten *et al.*, 1986)**, while other studies have proven that it has a significant impact on economic animals and their production, particularly in livestock, as mentioned by **(Alahmed, 2004; El-Azazy & El-Metenawy, 2004)**. The presence of myiasis in Oman was confirmed by **Spradbery *et al.* (1992),** and from a review of previous studies in this region, it was proved that the flies that cause myiasis in the area are endemic to the region.

In the same region, a researcher **(Abo-Shahada, 2005)** studied the screwworms of the type *Chrysoma bezziana* during the period from 1999 to 2000 and recorded the infection rates. The morphological description of the types of myiasis is very critical in identifying the different types, also to know the fine structures found on their surface that had been identified by scanning electron microscope (SEM).

This study aims to investigate the fine structure of flies larvae of *Chrysomya bezziana*genus, that cause myiasis in farm animals in Saudi Arabia. This study is the first in the region.

2. Materials and Methods

This study was conducted to classify and describe flies larvae of *Chrysoma bezziana*, that infect sheep of (*Ovis aries*) genus in the Eastern Province of Saudi Arabia during 2018. Larvae were collected for examination from local animals in the slaughterhouses in Al-Qassim region, from random samples. Larvae were collected from the infected places in the head area and from the nasal cavity of the animals, then they were placed in warm water until they died, they were placed after that in a 70% alcoholic glycerol solution. To prepare the samples for study with light microscope, the fly larvae were fixed by placing them in 70% ethyl alcohol, then they were permanently preserved in 70% alcohol with 5% glycerol added. They were photographed and permanent slides of the samples were made using **(Pritchard & Kruse, 1982)** method, while the preparations relevant to study of scanning electron microscope (SEM) were done according to **(Keirans et.al. 1976)** method, subsequently it was examined with an electron microscope and pictures of the fine structures were taken with an electron microscope (SEM) (JEOL-SEM-JSM-5600LV, Japan) to identify the classification status of the larvae that were collected during the study, with reference to the following taxonomic books: **(Smith,1973 Bland and Jaques,1980; Borror et. al., 1989; Zumpt 1960).**

3. Results and Discussion

3.1 Results

The study found *Chrysoma bezziana* species in the head and nasal cavity of infected sheep, and the present stages were the second and third larval stages, L2 and L3.The study also showed infection rates in Table 1 and Chart 1 in males and females, and females had a higher percentage than males, as the table and graph show.

As Table 2, 3 and Chart 2, 3 show, infection rates during the year, the most affected were spring and summer, and the least were in winter and autumn.

3.2 Classification

In sheep of the type Chrysoma bezziana, nasal myiasis falls under the classification.:
Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Family: Calliphoridae
Genes: Chrysomya
Species: *C. bezziana*

2-2-Key to 3rd Instars of *Chrysomyinae:*

1) Body without prominent papillae except few on posterior segment 1
2) Posterior spiracles virtually hidden in a deep cavity of the posterior segment...2
3) Posterior spiracles flush with face of posterior segment.....3
4) Posterior spiracle with peritreme complete (closed) or peritreme indistinct, button within peritreme.....4
5) Peritreme open, button indistinct and in open area of peritreme..... 5
6) Respiratory slits of posterior spiracles straight and roughly parallel……6
7) Tracheal trunks not heavily pigmented 8
8) Posterior margin of segment 11 with dorsal spines 9
9) Bands of black spines, thornlike with single teeth, not forming files anterior spiracle with 4-6 papillae ……..*Chrysomya bezziana*

3.3 Description

The body of the third-instar larva of Chrysoma bezziana, is usually of muscoid-shape that tapers anteriorly.When examined under a light microscope, the cephalopharyngeal skeleton, cephalopharynex, appears stiff and visible through the prothorax in the cephalic region of the body **(Figure1).**

The body of the larva is cylindrical in shape, very delicate and elongated. It contains 12 segments ranging in length from 3.10 to 2.16 mm, with width from 9.1 to 4.3 mm. The cuticle is transparent, tends to a pale yellow color and tends to be brown in some of the specimens in the third instar larvaL3.The body consists of 12 segments, the second of which contains a pair of anterior spiracles, mouth-hooks that are well-formed extending out from the body. The body wall contains protrusions at the ends, which contain well-formed spines at the ends of the segments, arranged in rows. **(Figure2)**

The cuticle in the area between the segments contains spines and also in the pro- and mesothorax, each has a darkened and recurved part toward the body **(Figure3)**

Scanning electron micrographs showed the cephalic segment composed of a pair of nipples adjacent to a pair of dome-

shaped maxillary palp. It is a pair of strong mouth-hooks, which is a broad area containing many oral grooves and a trilobed labium. Four to six papillae were observed on each anterior spiracle. The intersegmental spines between the pro- and mesothorax are very broad-based with each having a very sharply pointed tip that is recurved posteriorly. It is noticed in the thoracic segments in the ventral view that a pair of trichoid sensilla appear, which are composed of three setae. At the caudal segment, a pair of spiracles could be seen within a slightly sunken depression and are visible in the electron microscope. The dorsal ends of the inner and middle spiracular slits are slightly convergent, while those of the [outer] spiracular slits are slightly divergent. Using the SEM, it has been shown that the distances between the spiracular slits are not empty and contains trichoid sensilla and hairs around them **(Figure4.)**

3.4 Spines distribution

The number of spines on the dorsal side is less than the ventral side, the first segment is devoid of spines, while the second segment contains very few spines, and the middle segments contain (4-5) elongated, semi-regular rows containing an average of (200 spines), that are fine, small and conical in shape. It is also shown by the electron microscope that it is small and curved down and varies according to the place in terms of shape and thickness. (**Figure6,7)**

3.5 Mouth-hooks

The front segment of the body contains the mouth opening, which contains strong mouth-hooks inside it that are used for feeding and attachment. The hooks take a dark brown color and appear at the front a hidden and prominent simple part of it, and it is not clear by an electron microscope, but when examined under a light microscope it appears internal through the front end of the first segment, while its parts are visible under the cuticle. The two hooks are adjacent to the body's side lines. (**Figure 1, 2)**

3.6 Spiracles

The anterior spiracle is palmate in shape with 4 branches and 5 papillae arranged in a single row and is located at the dorso-posterior margin on each side of the prothorax, as it appears by light and electron microscopy **(Figure6,7)**. Anterior spiracles are clearly formed in the second segment of the body, taking a yellowish pale color, from which a group of forked tubes extend inside the body. It is compacted at the dorso-posterior margin on each side of the prothorax. In the caudal region at the end of the body, the posterior spines of the third instar larva are close together, with spaces at the dorsal, inner and middle end of the spiracles that are far apart from the other. There is a slit between them as in the figure.

The last segment of the body's segments contains two spiracles, the posterior spiracles are separate, close together and each is carried on spiracles tubes. The spiracles contain 3 clear chitinous slits, and the slit in the free, far side of it contains a small, delicate, circular-shaped spiracle. The spiracles are at a level lower than the surface of the body, as it appears as if they are located in a hole in the cavity of the posterior segment. (**Figure 4, 5)**

4. Discussion

By studying *Chrysomya* larvae, it is shown that the body is compressed from top to bottom, with nipple protrusions distributed on the body segments. The shape of the mouth opening is evident as a small wrinkled slit. The descriptive study showed the beginning of the formation of the sternum and the tergum, where the segment is divided into two halves and terminal parts, and the spines are micro in size with a pointed end, and the spiracles are at a low level at the surface of the body, and this is consistent with what **(Nahif and Madel, 1975, 1978)** mentioned. During the study of *Chrysomya* larvae, it was found that the body is cylindrical in shape and the hooks are conical with little protrusion, and the spines are broad in origin with a pointed tip, and the spines of the first segment have a collar around their ends. The spiracles are also closed lower than the surface of the body. The study showed the shape of the external structures of the anterior and posterior spiracles, and it is consistent with **(Leite and Guevara, 1993).** The study revealed structures and descriptions that had not been studied in the Kingdom of Saudi Arabia before.

Prior to the present study, morphological research of the immature stages of *C. bezziana* has been limited to that by **Kitching (1976)** where comparison of some morphological features was made with other flies in the genus *Chrysomya*. Previous studies showed many detailed characteristics of the third instar larva and puparium of *C. bezziana*. Previous studies of *C. bezziana* worms using light and scanning electron microscopy confirm this distinguished feature when compared with some of the other medically important fly species reported by **Sukontason *et al.* (2004).** The broad base and the sharply pointed recurved spines of *C. bezziana* differ greatly in appearance when compared to the other species recorded previously **(Sukontason *et al.* 2004, 2005)**. Both of these features of *C. bezziana* larvae are different from those of other species of flies **(Ishijima 1967; Kitching 1976; Sukontason *et al.* 2004; 2005)**. The shape of the oral grooves in the cephalic segment and the posterior spiracular hairs on the spiracular discs of the caudal segment of larvae differ also **(Sukontason *et al.* 2003a, b, 2004,2005).** Comparison of posterior spiracular hairs was also used by **Aspoas (1990)** as one of the characteristics used for distinguishing between morphologically similar larvae of flesh fly species. Observations from electron microscopy in this study provided some fine details of the morphology of the third instar larva of *C. bezziana* that may help explain some of the behavior of this species. For example, the feeding ability of *C. bezziana* larvae can be explained by the strong mouth-hooks that are used to penetrate deeply into many tissues. **Prasad et al. (2000)** noted that *C. bezziana* larvae can burrow into healthy tissues adjacent to wounds and may also penetrate into the bones surrounding the nasal sinuses. The other components, which were observed in the third instar larva are used as sensory organs such as antennae, maxillary palps, and trichoid sensilla. These structures were similar in comparison to other flies such as *C. megacephala*, *C. rufifacies*, and *C. nigripes* **(Sukontason et al. 2003a, b, 2004).** In studies of

fine structures of house fly larvae (*Musca domestica*) using transmission electron microscopy (TEM), the dome-shaped structure of the antenna was assumed to be an olfactory receptor (**Chu-Wang and Axtell 1971);** whereas, the maxillary palp may serve a dual function for both chemical and mechanical contact **(Chu-Wang and Axtell, 1972).**

The current study expands our knowledge of the fine details of the external morphology of the third instar larva of *C. bezziana*, a species found in many countries around the world, including Saudi Arabia. However, in this research, many morphological observations of the structures of larvae and their differences under light microscope were shown, as well as some of the different studies that were made clearly confirm this feature when compared with other medically important species of flies recorded by **Sukontason *et al*, 2008.**

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Table 1 Clarify the percentage of infection in sheep (males and females)

	Infected	Total			
Chrysomya bezziana	Male	Count	90	160	250
		%	20.0%	35.6%	55.6%
	Female	Count	50	150	200
		%	11.1%	33.3%	44.4%
Total	Count		140	310	450
	%		31.1%	68.9%	100.0%

Chart 1 Clarify the percentage of infection in sheep (males and females)

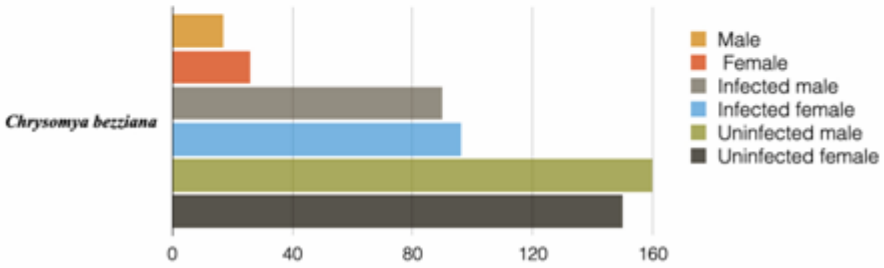


Table 2 - seasonal injury in stages of larval stages and determine the intensity of injury in each in Chrysomya bezziana larva (2017-2018)

Month	Infected	larva stage 1	larva stag 2	larva stage 3	Total
June	Count	1	0	5	6
	%	0.5%	0.0%	2.7%	3.3%
July	Count	0	5	8	13
	%	0.0%	2.7%	4.3%	7.1%
Agust	Count	0	3	5	8
	%	0.0%	1.6%	2.7%	4.3%
September	Count	2	3	10	15
	%	1.1%	1.6%	5.4%	8.2%
October	Count	0	10	15	25
	%	0.0%	5.4%	8.2%	13.6%
November	Count	1	6	4	11
	%	0.5%	3.3%	2.2%	6.0%
December	Count	0	5	4	9
	%	0.0%	2.7%	2.2%	4.9%
January	Count	0	2	20	22
	%	0.0%	1.1%	10.9%	12.0%
Fubrarary	Count	0	10	25	35
	%	0.0%	5.4%	13.6%	19.0%
March	Count	0	5	3	8
	%	0.0%	2.7%	1.6%	4.3%
April	Count	2	8	8	18
	%	1.1%	4.3%	4.3%	9.8%
May	Count	1	3	10	14
	%	0.5%	1.6%	5.4%	7.6%
Total	Count	7	60	117	184
	%	3.8%	32.6%	63.6%	100.0%

Chart 2 - seasonal injury in stages of larval stages and determine the intensity of injury in each in Chrysomya bezziana larva (2017-2018)

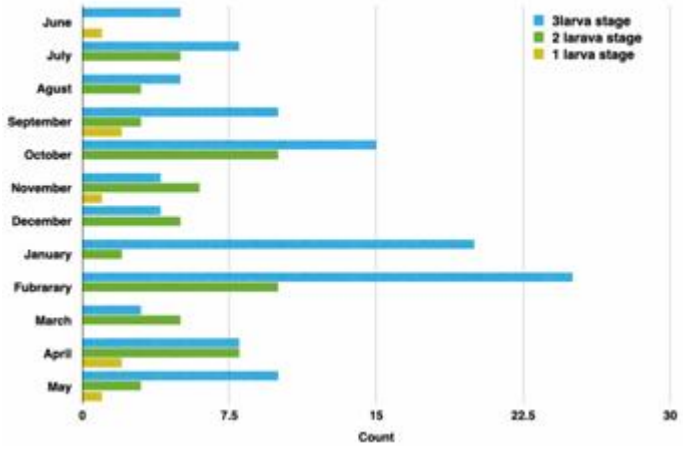
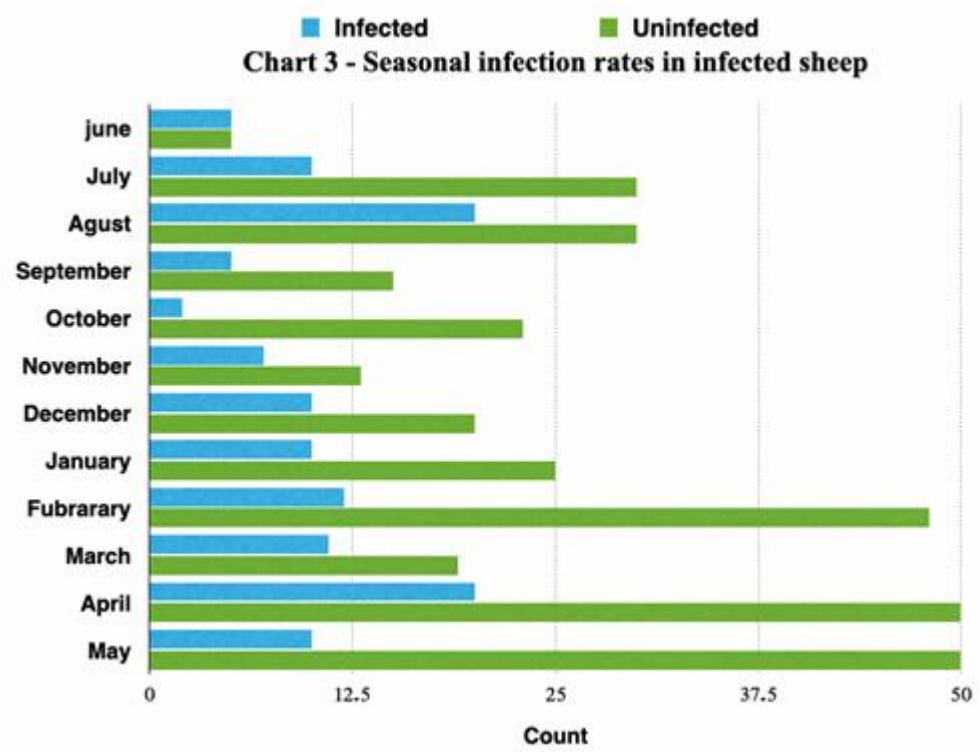


Table 3 - Seasonal infection rates in infected sheep

Month	Count	Infected	Uninfected	Total
June	Count	5	5	10
	%	1.1%	1.1%	2.2%
July	Count	10	30	40
	%	2.2%	6.7%	8.9%
Agust	Count	20	30	50
	%	4.4%	6.7%	11.1%
September	Count	5	15	20
	%	1.1%	3.3%	4.4%
October	Count	2	23	25
	%	0.4%	5.1%	5.6%
November	Count	7	13	20
	%	1.6%	2.9%	4.4%
December	Count	10	20	30
	%	2.2%	4.4%	6.7%
January	Count	10	25	35
	%	2.2%	5.6%	7.8%
Fubrarary	Count	12	48	60
	%	2.7%	10.7%	13.3%
March	Count	11	19	30
	%	2.4%	4.2%	6.7%
April	Count	20	50	70
	%	4.4%	11.1%	15.6%
May	Count	10	50	60
	%	2.2%	11.1%	13.3%
Total	Count	122	328	450
	%	27.1%	72.9%	100.0%



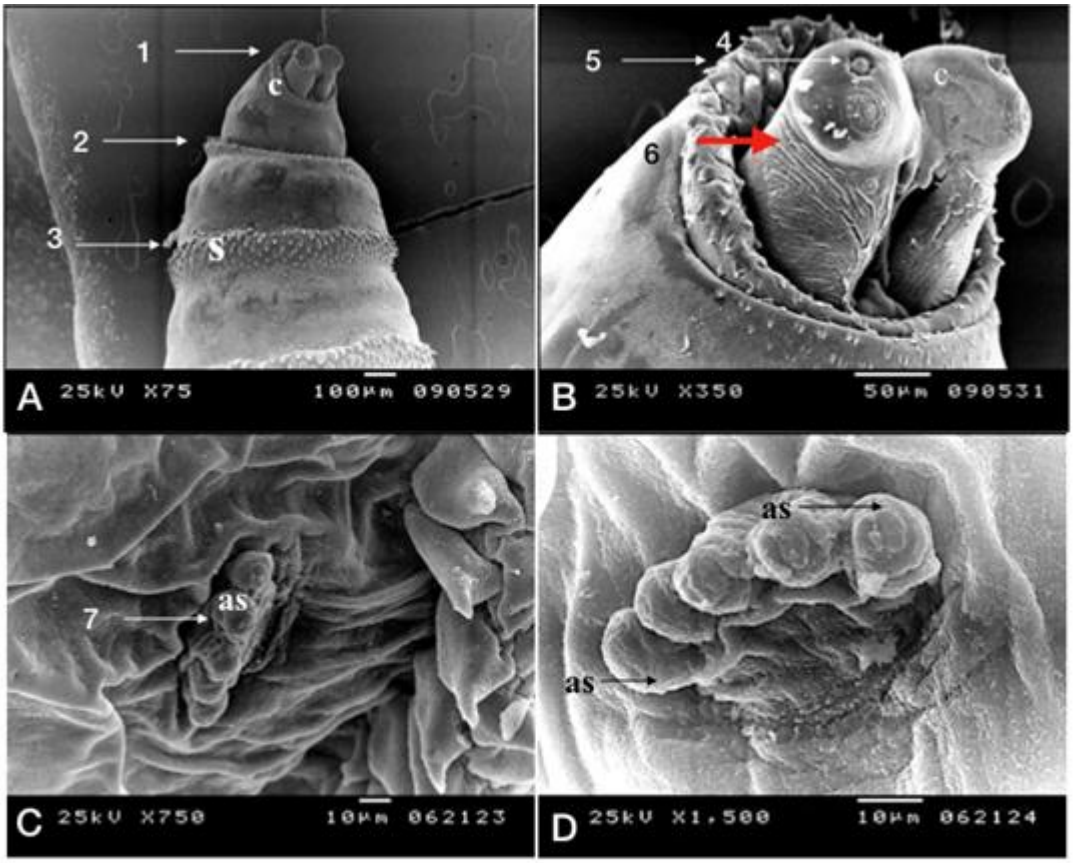


Figure. 1 Scanning Electron Microscope of third-instar larva of *Chrysomya bezziana*. Anterior region .(A,B,C,D) .
1- anterior part 2-3- spines between pro-and mesothorax (s)
4- antenna 5- spine Crowen 6- oral groove 7- Anterior spiracle showing palmate shape due to five papillae arranged in single row.

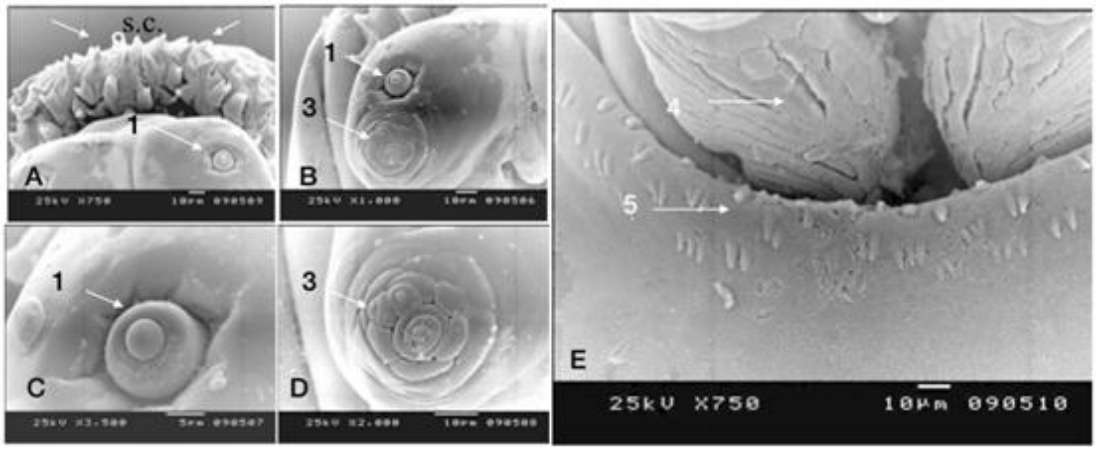


Figure. 2 Scanning Electron Microscope of third-instar larva of *Chrysomya bezziana*. Anterior region .(A,B,C,D,E) .
1- antenna 2- maxilla palp 5-spine crown(s.c.) under mouthparts. 5-oral groove 7-antennary lobes(a.l) 8-spine Crowen under mouthparts 9-oral groove.

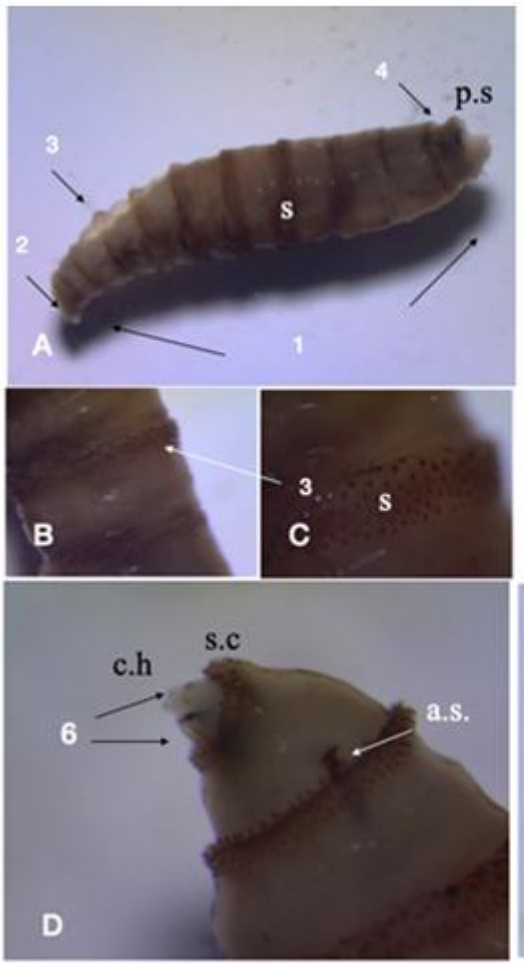


Fig. 3 Light micrograph of third-instar larva of *Chrysomya bezziana*. Anterior region . (A,B,C,D,E)cephalopharyngeal skeleton (c), anterior spiracle (as), and spines between pro- and mesothorax (s). Anterior spiracle showing palmate shape due to five papillae arranged in single row.

Abbreviation:

1-third-instar larva-2-spine crown (s.c.) - 3 spines between pro- and mesothorax (s)4-posterior spiracle (p.s.)6-cephalic hooks(c.h.) 7-antennary lobes(a.l) 8-

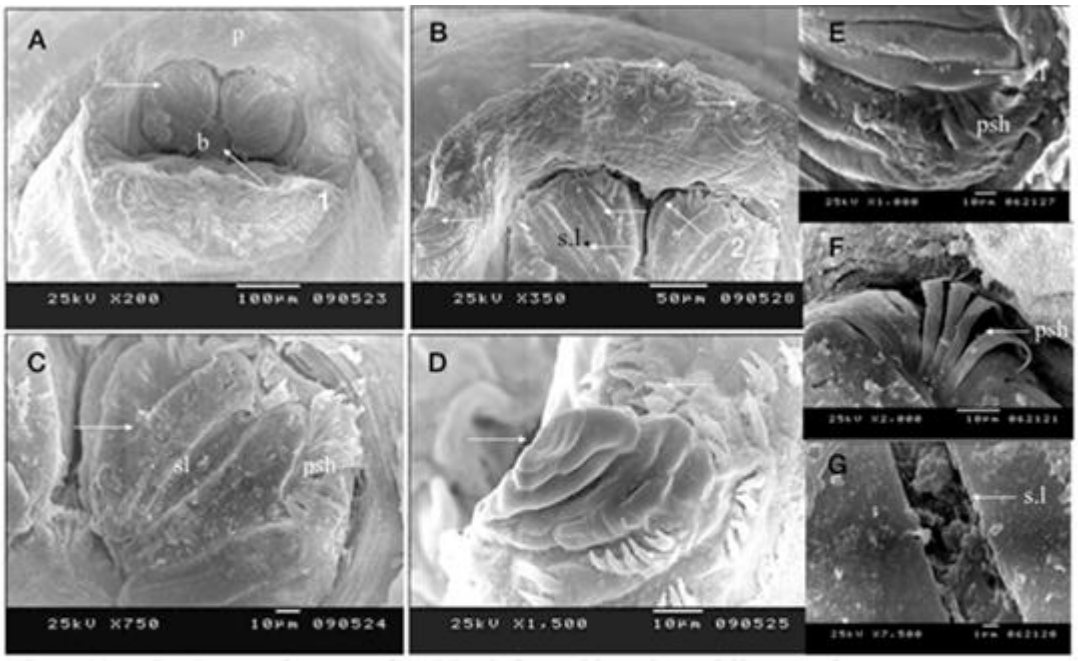


Figure. 4 Scanning electron microscope of caudal end of second-instar larva of *Chrysomya bezziana*.
A-Posterior spiracular discs each bearing three spiracular slits (s.l.) and fringed with posterior spiracular hairs (psh) around the outer periphery. B-Dorsal ends of inner and middle spiracular slits are slightly convergent, and that of the outer spiracular slit is slightly divergent. A small compressed button (b) is observed at the base of the slits. C-Cluster of broad and branched flattened spiracular hairs .Caudal view of contiguous posterior spiracles. Dorsal ends of the inner and middle spiracular slits (s.l.) are slightly convergent; whereas, that of the outer slits is slightly divergent. Peritreme (p) is thick and incomplete Cluster of broad and branched flattened spiracular hairs D- apple in the terminal of caudal area .

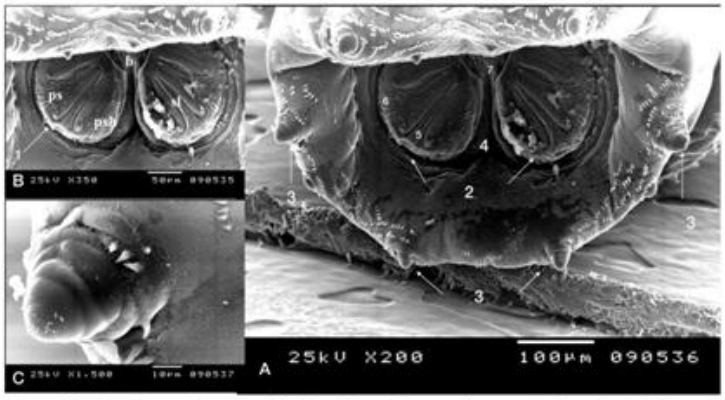


Figure. 5 Scanning Electron Microscope of third-instar larva of *Chrysomya bezziana*. Anterior region .(A,B,C) A-Dorsal ends of inner and middle spiracular slits are slightly convergent, and that of the outer spiracular slit is slightly divergent.B- the posterior part in large 1-2- around the spiracle 3- apple in terminal of end of th posterior parts 4- Dorsal ends of inner and middle spiracular slits are slightly convergent, and that of the outer spiracular slit is slightly divergent. A small compressed button (b) 5-Posterior spiracular discs each bearing three spiracular slits (sl) .6-Cluster of broad and branched flattened spiracular hairs (psh). observed at the base of the slits Caudal view of posterior spiracular discs each with the characteristic three spiracular slits (sl) with peripheral posterior spiracular hairs (psh) and basal compressed button (b) Posterior view of caudal segment showing positioning of posterior spiracles (ps)

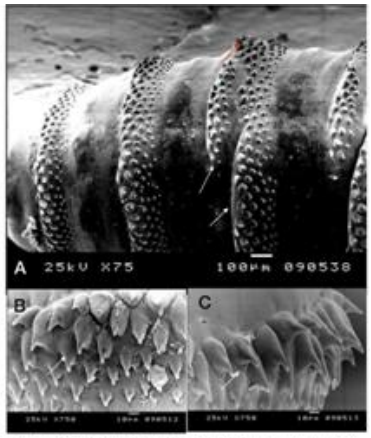


Figure. 6 SEM micrograph of third-instar larva of C. bezziana. (AB C) Enlarged view of intersegmental spines between pro- and mesothorax high- lighting their single, darkened and tapered tips that are recurved toward the body. Intersegmental spines between pro-and mesothorax showing their broad bases abruptly tapered to sharply recurved tips.

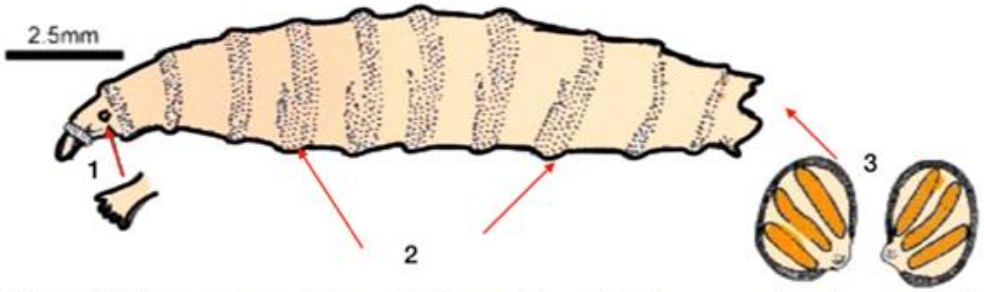


Figure 7.Diagram presents the third larval stage in *Chrysomya bezziana* myiasis. (1-Anterior spiracle -2- spine rings between the segments - 3-Posterior spiracle)