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Original Article

Light and scanning electron microscopy of *Gasterophilus intestinalis* (larvae and adult fly) infesting donkeys with emphasis on histopathology of the induced lesions.

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Abstract:

Stomach horse bots, the larvae of the *Gasterophilus intestinalis* flies (bot flies), inhabit the stomach of equines and cause damage ranging from mild gastritis to stomach ulcers and peritonitis. The adult flies are non-parasitic and incapable of feeding, yet they survive on the nutrients left from the larval stage for approximately 10-14 days, a period long enough for them to mate and lay eggs. In this study, morphological characterization of adult fly, second and third larval stages of *G. Intestinalis* (*Diptera: Oestridae*) is illustrated using light and scanning electron microscopes (SEM). The later clarifies the structures of cephalic segment, sensory array of distal maxilla (mouth hooks), thoracic and abdominal spines and terminal abdominal segment of second instar and third instar. Morphological differences between second and third stage larvae of *G. intestinalis* are also discussed in this work. Adult fly antennae and antennal sensilla are studied using SEM, each antenna consisted of scape, flattened disc like pedicel, pyriform funiculus and arista. Funiculus had five types of sensilla (trichoid, basiconic, coeloconic, clavate and auriculate). Arista was composed of one or two short basal segments without sensilla and one long distal segment covered with coeloconic III sensilla. It is concluded that light and SEM should be used in conjunction for the description of *G. intestinalis* but SEM is considered superior in description of their ultrastructure. Finally, pathological lesions in the stomach wall of infested donkeys are described.

Keywords: *Gasterophilus intestinalis*, Horse bot, donkeys, larvae, adult fly, antenna, sensilla, ulcer, Scanning electron microscope

INTRODUCTION

Gasterophilus species larvae (Diptera: Oestridae), commonly known as stomach bots, are obligatory parasites of the alimentary tract of equidae. Six species attacking domestic equidae were recorded by **Zumpt (1965)** and *Gasterophilus intestinalis* is the most common and world wide distributed species **(Kettle, 1995).**

Adult flies of *G. intestinalis* lay their eggs on the hairs of fore leg and shoulders. First stage larvae penetrate tongue and buccal mucosa after several weeks moult to second stage and reach to stomach. Third-stage larvae remain attached for about 8–10 months to the mucosa of the non-glandular portion of the host stomach, when mature pass in faeces and pupate in the ground **(Urquhart et al., 1996).**

During oviposition, adult female flies cause noticeable disturbance and irritation to the animals, rendering them difficult to control and liable to self-injury. Migration of first stage larva in mouth may cause pus pockets, loosened teeth, stomatitis, ulcers, pain during eating and loss of appetite. Second and third larval stages attach in clusters to the stomach wall by their well-developed mouth hooks (maxilla) and feed on tissue exudates by mandibles. Third-stage larvae provoke inflammation and sloughing of tissue with the formation of funnel-shaped ulcers surrounded by hyperplastic epithelium, leaving ring like swelling when the larva is removed. Heavy infestation by *G. intestinali*s may lead to sub-serosal abscess, peritonitis and eventually death may occur **(Kettle, 1995; Urquhart et al., 1996; Mullen and Durden, 2002 and Taylor et al., 2007).**

In spite of oral and gastric lesions caused by *Gasterophilus* larvae, there is little pathogenic evidence associating the infection with clinical illness and many animals support substantial populations of theses parasites without apparent illness. However, the ability of heavy infestations to cause stomach rupture, peritonitis and death of the animal underscores the significance of this disease **(Bowman, 2009).**

On rare occasions, *Gasterophilus* first stage larvae can penetrate the skin or eyes of humans causing cutaneous or ophthalmo-myiasis respectively. Human infestation occurs when there is direct contact between man and horse, for example, during horse grooming **(Zumpt. 1965; Harwood and James 1979 and Urquhart et al., 1996).**

In this study we describe the morphology of adult fly, second and third stage larvae of *G. intestinalis* infesting donkeys slaughtered at Giza Zoo in Egypt by the aid of light and scanning electron microscopes. Also we highlighted the morphology of adult fly antennae and antennal sensilla using scanning electron microscope. The pathological lesions in the stomach wall of infested donkeys were discussed.

MATERIAL AND METHODS

1- Parasites collection:

Larvae of *Gasterophilus intestinalis* (second and third stages) were collected at necropsy from the stomachs of donkeys slaughtered at Giza Zoo, Giza, Egypt to be fed to the carnivorous animals. Adult flies were obtained from laboratory rearing of third stages larvae according to the technique described by John and Petri (2006).

2- Parasites preparation for light and scanning electron microscope examination:

Larvae and adult flies were cleared in lactophenol, mounted on a clean glass slides on polyvol and left in the hot air oven at 50 °C to dry for 24 hours **(Soulsby, 1982)** for light microscope examination.

For scanning electron microscope analysis, second and third stage larvae were fixed immediately in 2.5% glutaraldehyde solution in 0.2 M sodium cacodylate buffer (pH 7.2) for 4 hours at 4°C, followed by post fixation in osmium tetreoxide (OsO_4) for 2 hours then rinsed 3 times in sodium cacodylate buffer, dehydrated in ascending graded ethanol then dehydrated using Critical Point Dried instrument with liquid carbon dioxide, mounted on cupper stubs with double-sided adhesive tape, coated with gold using S 150A Sputter Coater- Edwards- England (Rufz-Martinez *et al.,* 1989). Head of adult fly and antenna were separated by clean sterilized fine needle, mounted on cupper stubs with double-sided adhesive tape then coated with gold using the same Sputter coater previously mentioned **(Zhang *et al.,* 2012 and Zhang *et al.,* 2016).** Finally, the specimens were observed and photographed digitally using scanning electron microscope Quanta FEG250, European Union, operated at20 kV in the National Research Center, Dokki, Egypt.

3- Histopathological examination:

The collected tissue specimens from infested stomach walls were fixed in 10% neutral buffered formalin then routinely processed, paraffin embedded, sectioned into 5 µm thick sections and stained with hematoxylin and eosin and sections were examined using light microscope **(Bancroft and Stevens, 1996).**

RESULTS

Visual and light microscope identification

Second stage larvae were grub-like and 10-14 mm in length. Their body segments were provided with three rows of spines except the last two segments carrying only two rows. Mouth hooks (maxillae) were saddle like excision before genticulate bend. Mandibles were characterized by their serrated edges. The terminal abdominal segment carried two spiracular plates and lateral warts. Each spiracular plate had two slightly curved slits. These slits had 16-20 transverse bands (Fig. 1).

Third stage larvae were 15-20 mm long, reddish in colour, cylindrical in shape, tapered anteriorly and broad posteriorly. Anterior end had pair of maxillae, mandible and antennal lobe. Anterior spiracle had three finger-like papillae. All body segments beared two rows of spines, while the last two/three segments beared only one row of medially interrupted spines. Spines were slender with blunt tips. Posterior spiracles consisted of two C-shaped spiracular plates and united along their inner margin. Each spiracular plates had three curved slits (Fig.2).

Pupa was brown in colour with ill-defined transversely placed dark spots. It carried two pupal horns anteriorly and the plates,typically shaped of third larval stage body spines were clearly visible. It measured 10mm length x5mm width (Fig. 3).

After 18-21 days at room temperature, adult flies emerged from pupae. The adult fly resembled honey-bee in appearance and measured 10-14mm in length. The head was broader than long with small facets, bearing two antennae inside grooves. The thorax was black-brown in colour with dark yellow hairs. The abdomen was curved ventrally and yellow in colour with six ill-defined transversely located dark spots. The antennae were dark yellow in colour and each consisted of three segments and bare arista. The wings had wide apical cell and dark areas in the whole width of the wing at the middle and two dots at the wing apex. The legs were slender and yellow brown in colour. Trochanter of hind leg showed a spatulate process and a tubercle in male and female, respectively.1st tarsal segment in male had normal width and about half length of the tibia (1.14mm / 2.28 mm), while in females, it appeared dilated and the length of 1st tarsal was more than that of males (1.33 mm/ 2.1mm) (Fig. 3).

Scanning electron microscopy (SEM):

Third larval stage (L3):

The cephalic segment was small and had a pair of maxillae, pair of mandibles, two antennal lobes and small masses of simple spines existed ventral to each cephalic lobe. Mandibles were large and well developed and characterized by serrated lobes on the dorsal portion. Maxillae (mouth hooks) consisted of rhomboid shaped proximal part which was directly connected to the larval body and devoid of sensory structure and elongated cone shape distal part which had a sensory array. Sensory array included porous dorsum, polygonal plate, two rows of angle plates separated by ventral bands, shield tip at apex and shallow pits in distal portion of polygonal plate contain peg-like sensilla. Antennal lobes located dorsal to maxillae on the cephalic segment each lobe beared cephalic sensillae included olfactory sensillum (dom-shaped), gustatory sensory (clusters) and accessory peripheral sensilla (Fig. 4).

First thoracic segment was not extended in a shelf like manner over the cephalic segment. Thoracic and abdominal segments were provided with double rows of unequal spines (inverted drop with sharply pointed end) (Fig. 5).

The terminal abdominal segment was characterized by presence of two spiracular plates in the respiratory cavity. Each spiracular plates had three slightly curved longitudinal slits. Six cuticular sensillae were also located on the terminal abdominal segment. Four sensillae situated dorsal to spiracle plate. Other two sensillae were compound sensillae found on the lateral ventral lobes (lateral warts). The compound sensillae consisted of two trichoid sensillae and lateral pit-like sensillum (Fig. 6).

Second larval stage (L2):

The 2nd stage larvae resembled 3rd stage larvae but differed in absence of shallow pits, peg like sensillae and ventral bands on distal maxillae. The body segments carried three rows of spines. Mandible apical projections were more prominent than L3. Terminal abdominal segment had lateral warts

bearing trichoid sensilla and probable ecdysial scar between spiracular plates (Fig.7).

Antenna of adult fly:

A pair of antennae was located in the centre of the head frontal region between compound eyes. Each antenna composed of scape, pedicel, funiculus and arista (Fig. 8). The scape was the smallest triangular shaped segment has smooth surface without microtrichia. Pedicel was the second flattened disc like segment with microtrichia on cuticular surface. Scape and pedicel were covered with straight longitudinally grooved bristles of varying lengths called mechanoreceptors. Also, there was a single pedicellar button at the articular surface near the pedicellar cleft and it consisted of a circular dome centrally and a slightly convexing peripherally (Fig. 9).

The funiculus was the largest pyriform shaped segment, densely covered by microtrichiae (Fig. 10 and 11) and had five types of sensilla (trichoid, basiconic, coeloconic, clavate and auriculate). It was provided with large number of cave-like depressions called sensory pits (Fig. 8). Trichoid sensilla are considered the largest ones. They had slender shaft with tapered blunt tip. Basiconic sensilla were shorter than trichoid sensilla and classified according to their size into two subtypes (BaI and BaII); both of them were digitiform with elongated shaft and abruptly blunt tip, but BaI was longer than BaII. Clavate sensilla had characteristic spatulate appearance or club like resulted from a sub apical dilatation. They tapered abruptly with rounded broad tip. They distributed either singly on the surface or clustered in pits (Fig. 10). Auriculate sensilla was tapered from the base gradually and was nearly similar to rabbit ears. They were distributed either singly on the surface or in groups inside sensory pits. Coeloconic sensilla are considered the shortest ones and hvae two subtypes (CoI and CoIII); CoI was short peg with longitudinally deep grooves at distal end, while CoIII had smooth cuticular surface and clustered inside shallow depressions only on the arista. (Fig.11). Measurements of sensilla were mentioned in table 1. Arista had one or two short basal segments without sensilla and one long tapered gradually distal segment covered with coeloconic III sensilla (Fig. 8 and12).

Histopathological changes:

Macroscopically, the examined stomachs revealed a large number of bot fly larvae attaching mainly to the non-glandular portion of the stomach near the margoplicatus. Numerous distinct crater-like lesions with raised edges and deep centers were observed at sites of attachment of the larvae. While some of the larvae were detached at time of examination, some others were still firmly attached to the stomach wall with their anterior parts burrowed deeply into the mucosa.

Histopathologically, circum-scribed ulcers with complete loss of the lining mucosa and exposure of the submuscosa were prominent. The epithelium at the periphery of the ulcerative mucosa showed marked hyperplasia and hyperkeratosis. The submucosa showed areas of necrosis and deeper areas of intense fibrous

connective tissue proliferation with occasional newly formed blood vessels. Hyalinization and thickening of the submucosal blood vessels walls was observed. Multifocal infiltrations of inflammatory cells including neutrophils, macrophages and lymphocytes were seen together with intense infiltration of eosinophils at some regions (Fig. 12).

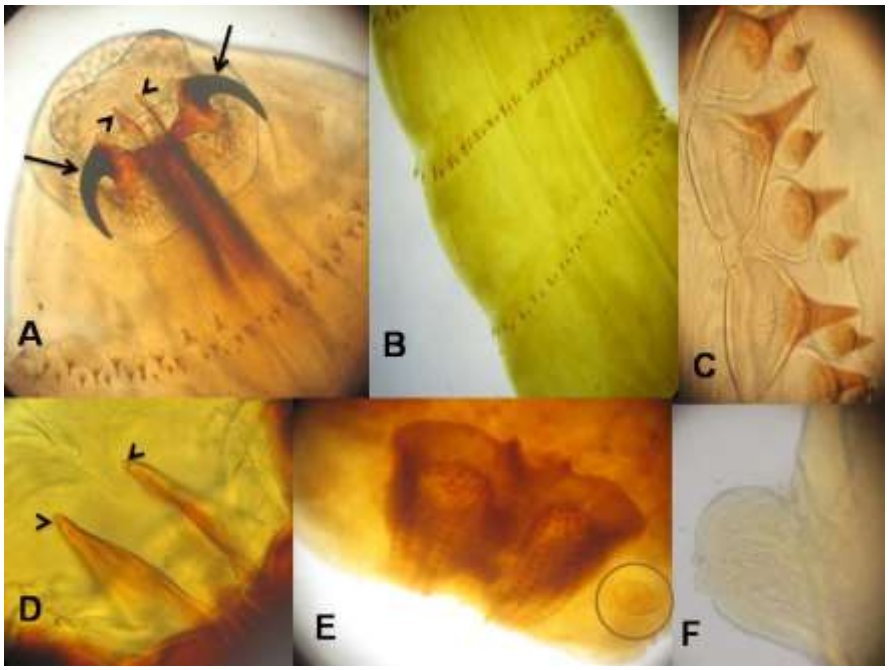

Fig. 1: Second instar of *Gasterophilus intestinalis*; A: anterior end (arrows, maxillae; arrow heads, manibles); B&C: spination pattern; D: mandibles (arrow heads); E: terminal abdominal segment with spiracular plates and lateral warts (circle) and F: higher magnification of lateral wart (digital camera).

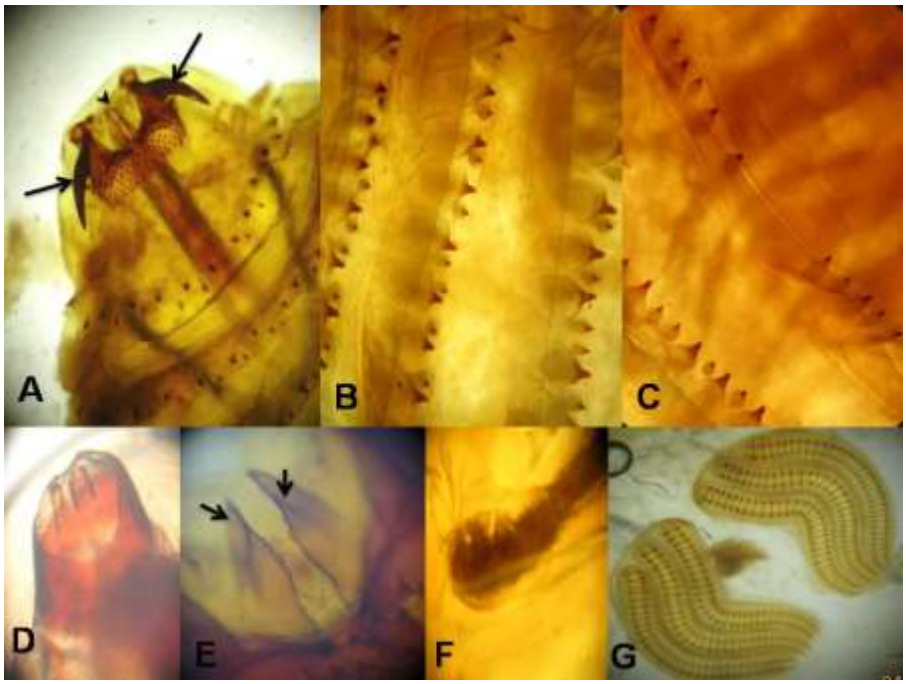

Fig. 2: Third instar of *Gasterophilus intestinalis*; A: anterior end (arrows, maxillae; arrow head, manibles); B&C: spination pattern; D: antennal lobe; E: mandibles (arrows); F: anterior spiracle and G: terminal abdominal segment with spiracular plates (digital camera).

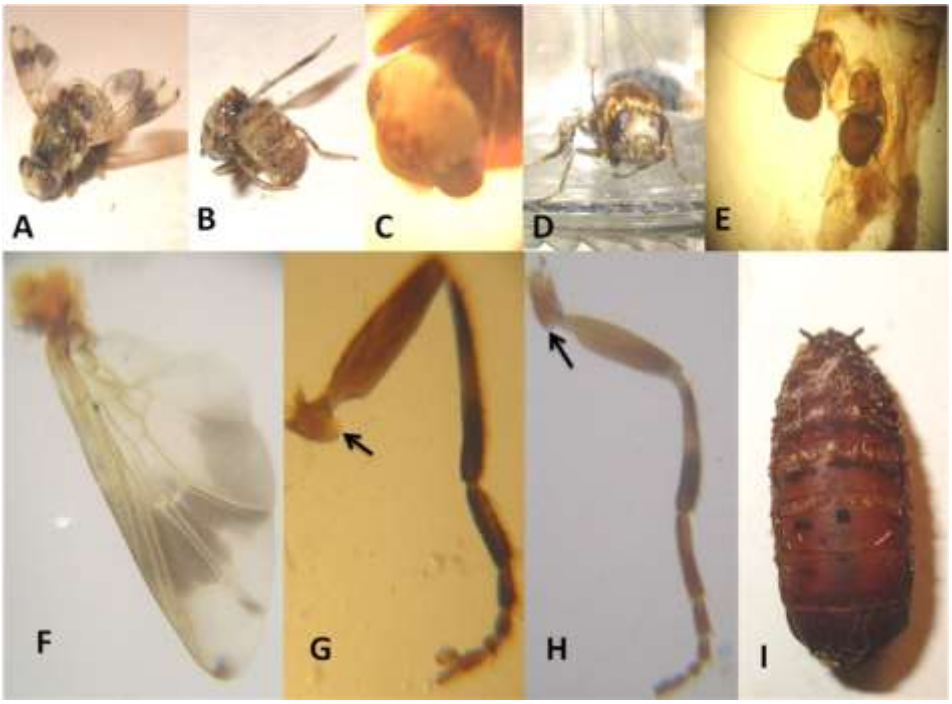

Fig. 3: Adult and pupa of *Gasterophilus intestinalis*; A& B: Adult fly; C: Head (ventral view); D: Thorax with yellow hair; E: Antennae; F: Wing; G: Male hind leg (Black arrow: Trochanter process); H: female hind leg (Black arrow: Trochanter tubercle) and I: Pupa (digital camera).

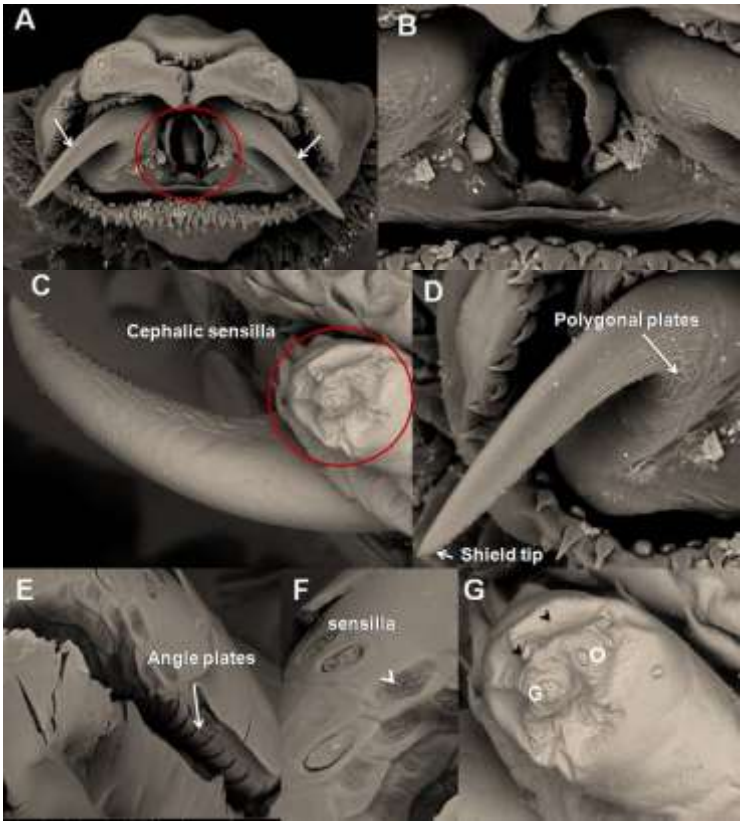

Fig. 4: SEM of 3rd larval stage cephalic segments and maxilla**;** A: cephalic segment with antennal lobes, pair of laterally directed maxillae (white arrow) and medially directed mandibles (red circle) (600 X); B: mandibles (1200 X); C: antennal lobe (red circle) and laterally directed maxilla (800 X); D: polygonal plates of maxilla with shield tip (1500 X); E: angle plates of maxilla (2000 X); F: shallow pits with peg-like sensilla (4000 X) and G: antennal lobe bearing dom-shaped olfactory sensillum (O), gustatory sensory cluster (G) and accessory peripheral sensilla (black arrow heads) (1200 X).

Fig 5: SEM of 3rd larval stage thoracic and abdominal; A: cephalic and thoracic segments (80 X) and B: spination pattern (200 X).

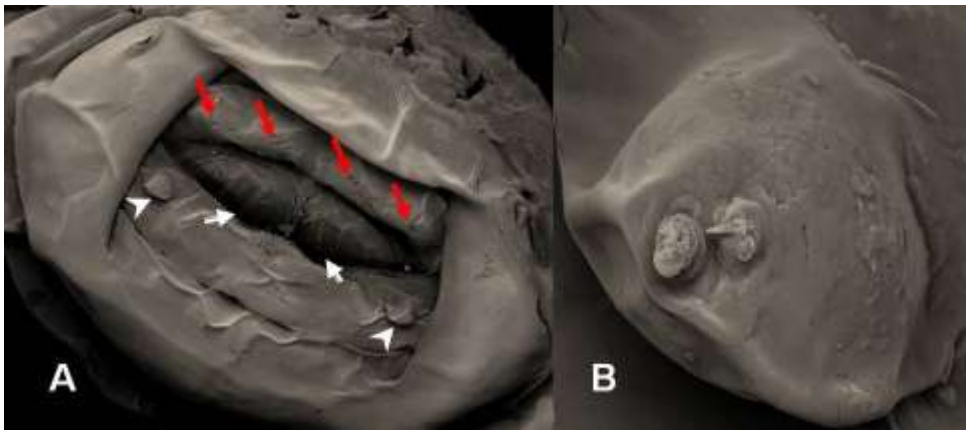

Fig. 6: SEM of terminal abdominal segment of 3rd instar; A: Respiratory cavity showing spiracular plates (white arrows), lateral warts (white arrow heads) and four individual sensilla (red arrows) (100 X) and B: lateral warts bearing two trichoid sensillae and lateral pit-like sensillum (1600 X).

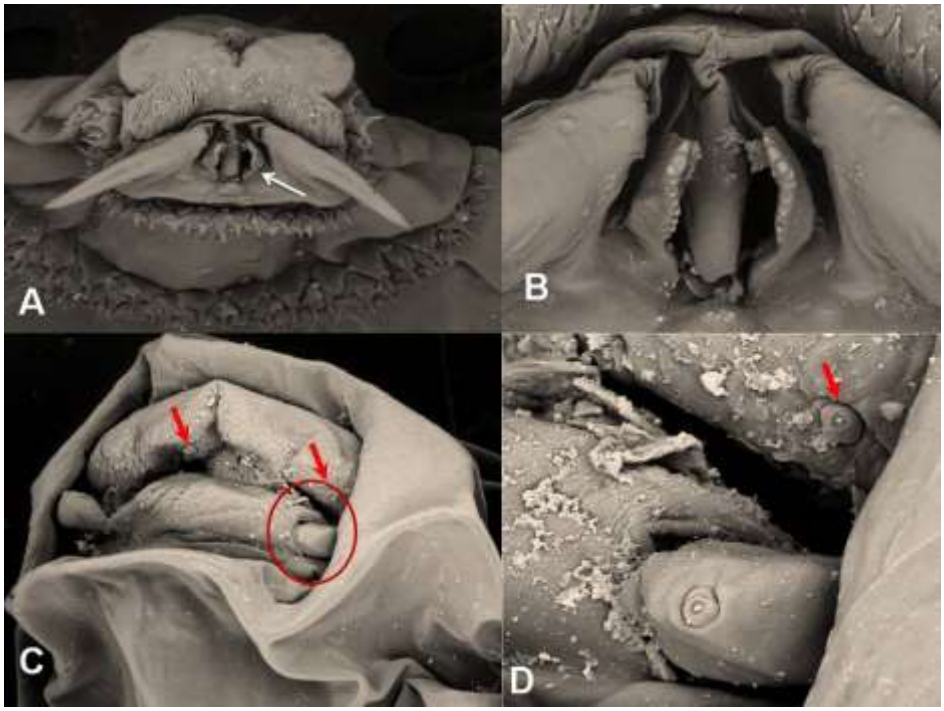

Fig. 7: SEM of 2nd larval stage of *Gasterophilus intestinalis*; A: cephalic segment with antennal lobes, maxillae, mandibles (white arrow) and spination pattern (400 X); B: mandibles (1600 X); C: Terminal abdominal segment with lateral warts (red circle) and individual sensilla (red arrows) (400 X) and D: lateral wart bearing trichoid sensilla and individual sensilla (red arrow) (1500 X).

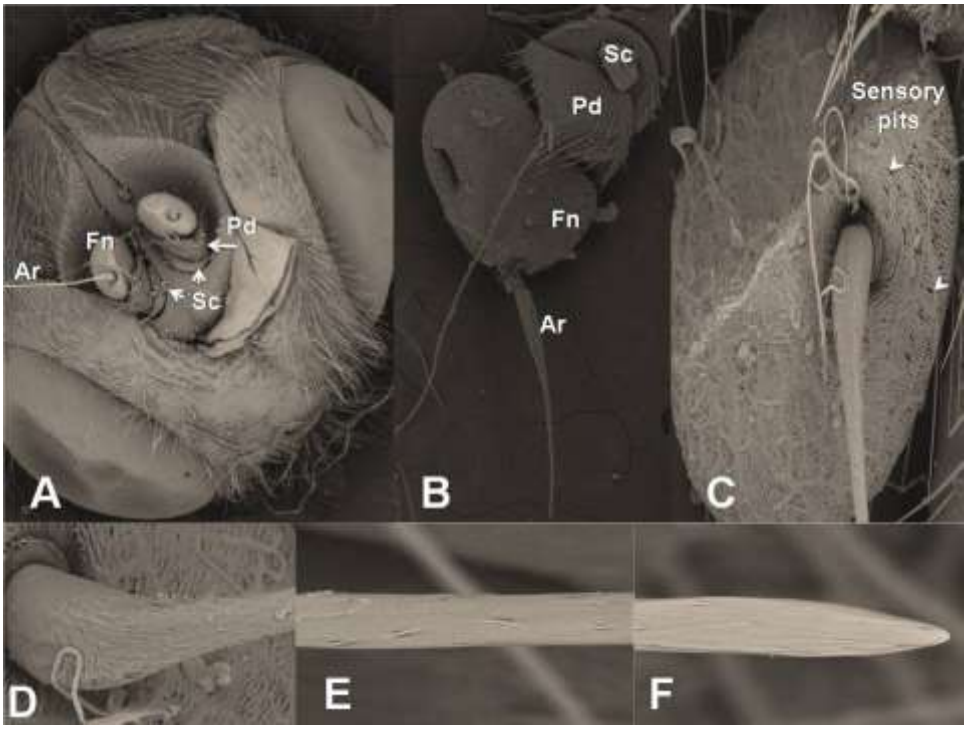

Fig. 8: SEM of *Gastrophilus intestinalis* head A: Antennae in frontal region of the head (100 X); B: antenna (X 200); C: antennal funiculus with sensory pits (white arrow heads) (1000 X) and D-F: base, middle and tip of arista (D=2000 X, E= 4000 X and F= 5000 X). Abbreviations: Ar: arista; Fn: funiculus; Pd: pedicel and Sc: scape.

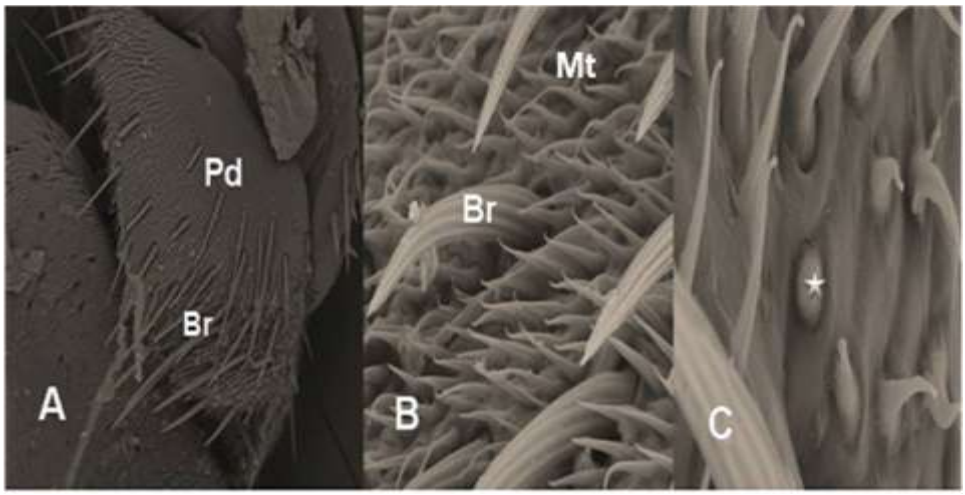

Fig. 9: SEM of pedicel; A: Pedicel (Pd) covered with longitudinally grooved bristles (Br) and microtrichiae (800 X); B: bristles (Br) and microtrichia (Mt) on pedicel (6000 X) and C: pedicellar button (white star) (16000 X).

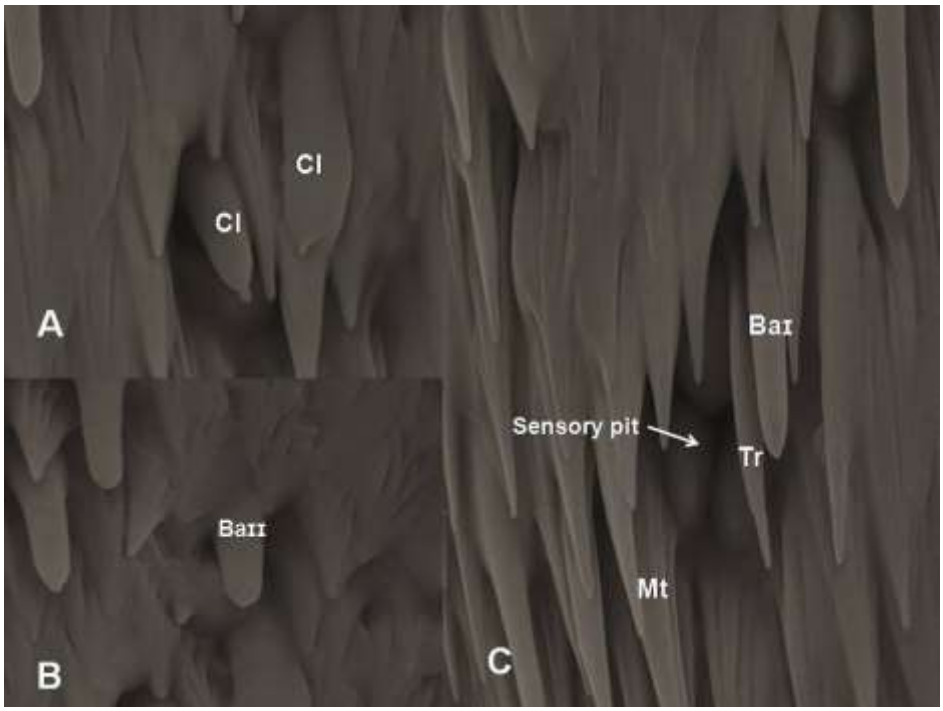

Fig. 10: SEM of sensilla on antennal funiculus; A: clavate (Cl) sensilla (24000 X); B: basiconicII (BaII) sensilla (12000X) and C: sensory pit covered with microtrichia (Mt), basiconicI (BaI) and trichoid (Tr) sensilla (16000 X).

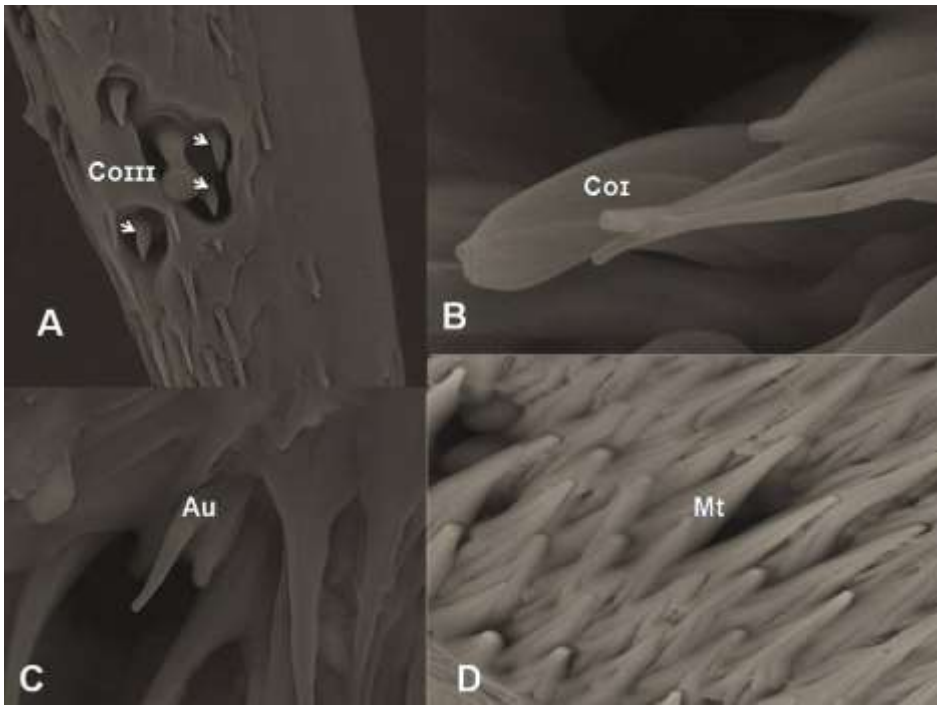

Fig. 11: SEM of sensilla on antennal funiculus; A: basal part of arista equipped with Coeloconic III (CoIII) sensilla (white arrows) (6000 X); B: Coeloconic I (CoI) sensilla (24000 X); C: auriculate (Au) sensilla (300000 X) and D: microtrichia (Mt) (16000 X).

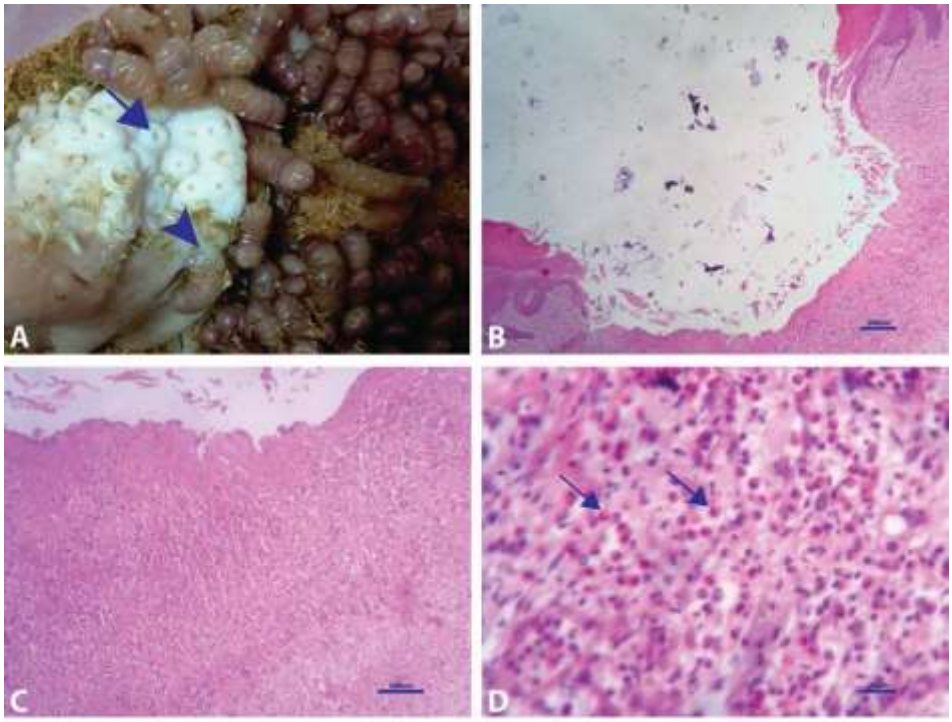

Fig. 12: Ulcerative gastritis caused by G. intestinalis larvae in the non-glandular stomach of a donkey; A: Several bot flies larvae attaching to the non-glandular portion of the stomach wall near the margoplicatus. When attached to the wall, the anterior part of the larva is completely burrowed in the mucosa (arrow head) and when detached, the attachment site shows a crater-like ulcer with raised edges and deep centers (arrow); B: The lesions are represented by deep ulcers involving complete loss of the mucosa and thickening of the adjacent intact epithelium (Bar=200 µm); C: Intense fibrous connective proliferation underlies areas of necrosis (Bar=100 µm) and (D) Multifocal infiltration of eosinophils (arrows) are prominent throughout the stomach wall (Bar=20 µm).

Table1: Morphometric measures of funiculuar sensilla in *Gastrophilus intestinalis*.

Sensilla	Length (µm)	Base diameter (µm)	Tip diameter (µm)
Trichoid	12.23	2.21	—
Basiconic I	8.19	2.25	—
Basiconic II	6.85	1.55	—
Coeloconic I	4.31	1.84	—
Coeloconic III	5.28	1.66	—
Clavate	12.25	1.54	2. 01
Auriculate	4.23	2.21	—

Discussion

True gastric myiasis in equines is caused by *Gasterophilus species. G. intestinalis* larvae remains attached to stomach mucosa for 8-10 months. In our study they were collected from the non-glandular region of the donkey's stomach at necropsy which is considered the predilection site for *G. intestinalis* as mentioned by **Soulsby (1982), Kettle (1996) and Urquhart et al. (1996).** Morphological characteristics obtained by light microscopical examination for both second and third stage larvae and the adult fly morphological features inspected in this study were similar to that described by **Zumpt (1965), Soulsby (1982), Kettle (1995), Urquhart et al. (1996), Taylor et al. (2007), Bowman (2009) and Williams (2009).**

The adult fly emerged from the pupal case after 18-20 days at room temperature, this investigation is nearly similar to **Harwood and James (1979), Kettle** (**1995), Urquhart et al. (1996), Mullen and Durden (2002), Bowman (2009) and Williams (2009).** Our results revealed that the pupal morphological characterization was brown colour with two pupal horns anteriorly represented the protrusion of anterior spiracles of the third stage larvae and typically shaped third stage larvae body spines, which is in accordance with investigations by **Zumpt (1965) and Principato and Tosti (1988).**

Regarding the scanning electron microscope, morphological observation, the cephalic segment contained pair of maxillae, pair of mandibles and two antennal lobs dorsally with cephalic sensillae. Distal part of maxillae was characterized by the presence of well-developed sensory arrays, variations in those sensory arrays were considered the most important feature for differentiation between different *Gasterophillus* species 3rd stage larvae as observed by **Cogley (1999), Cowell et al. (2007) and El-bakery and Fadly (2014). Zumpt (1965)** differentiated between the species using the curvature and base structure of maxillae. **Cogley (1999)** suggested that the function of the sensory array on distal maxilla surface including the shallow pits with sensillae is finding site of attachment to stomach wall while **Cowell et al. (2007)** mentioned that absence of these structures from the maxillae of some other species raises doubts about the function of sensillae in the pits and pointed out that the location of the cephalic sensillae (olfactory and gustatory sensillae) compressed near the host tissue would transmit much information to the larvae and thus has the potential to play the role of finding the attachment site. In regard to the thoracic and abdominal segments, our study illustrated the absence of first thoracic segment shelf pattern and presence of two rows of

unequal spines on body segment. Also, terminal abdominal segment was characterized by presence of two spiracular plates, four individual sensilae dorsally to spiracular plates and two lateral warts which carried two trichiod sensillae and lateral pit like sensillum. These findings were similar to those described by **Cowell et al. (2007) and El-bakery and Fadly (2014). Zumpt 1965** detected lateral warts on terminal abdominal segment, while **Cowell et al. (2007) and El-bakery and Fadly (2014)** illustrated different types of sensilla carried on warts using SEM as described in this study. As discussed by **Principato (1988) and Principato and Tosti (1988)** the posterior spiracles were situated inside the respiratory cavity enclosed by dorsal and ventral lips formed by cuticle which acted as a fence against inverse circumstances as inflammatory exudates.

Furthermore, the obtained results showed the differences between second and third stage larvae where the second stage larvae resembled the third stage larvae except for lacking ventral bands, shallow pits and peg-like sensilla on the distal part of maxilla. The mandible apical projection serrations were more prominent in the second stage larvae. The body segments of 2nd stage larvae provided with three rows of unequal spine. The terminal abdominal segment with two spiracular plates each contained two spiracular slits and ecdysial scar between two spiracular plates was only observed in the second stage larvae. These findings are similar to those mentioned by **Cogley (1999)** who studied the morphology of maxilla of second and third stage larvaes of *G*. *intestinalis* and **El-bakery and Fadly (2014)** whom recorded the difference between second and third stage larvae of *G. haemorrhoidalis* using scanning electron microscope.

This study also focused on observation of adult fly antenna. Sensory organs on the antennae of insects play an important role in locating mates, hosts, habitats and oviposition sites. As observed by **Cogley and Cogley (2000),** adults of *G*. *intestinalis* take less than one week to locate mates and hosts. Therefore, acute sensory capabilities can noticeably improve completing the fly life cycle **Zhang et al. (2012).** Antenna is considered the most complicated sensory organ in the adult fly. It is composed of scape, pedicel, funiculus and arista. Funiculus had different types of sensilla (trichoid, basiconic, coeloconic, clavate and aurticulate). These characteristics are similar to those described by **Zhang et al. (2016).** Also, **Zhang et al., (2012)** recorded that the funiculus of *Gasterophilus nigricornis* carried large number of multiparous trichoid, basiconic and auriculate sensilla and fewer numbers of clavate sensilla. These sensilla in *G. nigricornis* may be used to identify short-range sex pheromones in precopulatory courtship and are likely to be

concerned in the finding of equine kairomones before oviposition on horse hairs. This is established by conclusion in a behavioural study of *G. intestinalis* **(Cogley and Cogley, 2000)** which suggest that piles of horse dung are used as mating sites by newly hatched flies and that the kairomones that attract flies to lay eggs are probably secreted by horses.

The histopathological lesions observed in the stomach wall of the infested donkeys are consistent with those recorded previously in other equine species **Sołtysiak et al. (2014)** and are majorly caused by the direct physical damage caused at site of attachment of the larvae to the mucosa and the accompanied inflammatory reaction.

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الملخص العربي

الوصف الظاهرى باستخدام الميكرسكوب الضوئى والالكترونى ليرقات وذبابه الجاستروفيلس انتيستيناليس التى تصيب الحمير مع توضيح التغيرات الباثولوجية الناتجه عن الاصابه

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تسكن يرقات الذبابة المعوية (*Gasterophilus intestinalis*) معدة الخيول والتى تتسبب فى أضرار تتراوح بين التهاب المعدة الخفيف إلى قرحة المعدة والتهاب الغشاء البطنى لذلك فان هذه الدراسة قد قامت بالتوصيف الشكلى لليرقات من الطور الثانى والثالث وايضا الذبابة البالغة باستخدام الميكرسكوب الضوئى والالكترونى كما تم توضيح الاختلافات بين الطور الثانى والثالث من حيث المقطع الرأسي والخطاف الفمى والأشواك الموجوده على جسم اليرقة والمقطع البطنى النهائى منها. كما تعد هذه الدراسه هى الاولى من نوعها التى توضح الفرق بين الطور الثانى والثالث من اليرقة للذبابه المعوية واوضحت ايضا الوصف الشكلى للذبابه البالغه باستخدام الميكروسكوب الضوئى كما انه قد تم التوصل الى الوصف الدقيق لمختلف الشعيرات التى تحملها قرون الاستشعار الخاصه بالذبابه البالغه باستخدام الميكروسكوب الالكترونى . بينما اوضحت الدراسه ان استخدام الميكرسكوب الالكترونى يوضح التفاصيل الدقيقه الخاصه بكل من الذبابه البالغة والطور الثانى والثالث من اليرقات وتوصى باستخدام كل من الميكرسكوب الضوئى والالكترونى سويا للوصول الى صوره مكتمله عن شكل اليرقات والذبابه البالغه هذا وقد اظهرت الدراسة التغيرات الباثولوجيه التى تسببها يرقات الذبابه المعويه فى جدار معده الحمير.