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Dept. of Parasitology,
Fac. of Medicine, Assiut University.

MEGASELIA SCALARIS (DIPTERA: PHORIDAE) AS A CAUSATIVE AGENT OF HUMAN INTESTINAL MYIASIS: LIGHT AND SCANNING ELECTRON MICROSCOPICAL STUDIES

(With IV Plates)

By

MAHA S.I. SHAHEEN; RASHA A. HASSAN, and A.A. SAKLA

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ميجاسيليا سكالاريس (دايبترا:فوريدي) كعامل مسبب لتدويد الأمعاء في الإنسان : دراسة بالميكروسكوب الضوئي والإلكتروني

مها سيد ابراهيم شاهين ، رشا عبد المنعم حسن ، عاطف عطا الله سكلا

إن ذبابة الميجاسيليا سكالرايس ذات الظهر المقوس حشرة لها أهمية طبية على مستوى العالم. في دراسة معملية على عينات براز بها يرقات مجهولة تم إحضارها إلى (قسم الطفيليات – كلية طب أسيوط) تم فحص هذه العينات بواسطة الميكروسكوب الضوئي والإلكتروني للتعرف على نوع اليرقات كذلك تم توصيفها و تمت تنمية اليرقات على البراز في المعمل حتى وصلت إلى الطور البالغ كذلك تمت دراسة مراحل التطور من يرقات وعذارى و ذبابة بالغة. وتم عمل مسح إلكتروني لسطح اليرقات بواسطة الماسح الإلكتروني مع توضيح الجزء الدماغي وفتحات التنفس. واستنادا إلى السجلات السابقة المتاحة يعد هذا العمل هو العمل الأول في مصر الذي يسجل الميجاسيليا سكالاريس كعامل مسبب للتدويد بالأمعاء في الإنسان كذلك هو الأول من نوعه الذي يوضح تفاصيل المسح الإلكتروني لهذه اليرقات.

SUMMARY

Megaselia scalaris, the humped-back fly, is an insect of medical importance worldwide. In a laboratory-based study, stool samples with a queery maggot infestation were brought to the Department of Parasitology, Faculty of Medicine, Assiut University. Samples were examined and the presence of maggots was confirmed. Light and scanning electron microscopy (SEM) were used for identification of

these maggots. Larvae were allowed to develop into adults by being reared on a stool culture. The larvae and the flies were identified as *Megaselia scalaris* using standard keys. Life stages of *Megaselia scalaris:* larva; pupa; and adult fly were described and their morphological features were reported. Surface ultrastructure of the third-instar *Megaselia scalaris* was described using scanning electron microscopy, with the cephalic segment, and posterior spiracles being emphasized. Depending on the available records, the present work is considered the first record of *Megselia scalaris* as a causative agent of myiasis in Egypt. It is also the first work that explains the details of surface ultrastructure of third instar of *Megselia scalaris* in Egypt.

Key words: *Megaselia scalaris, Human Intestinal Myiasis, Microscopical Studies*

INTRODUCTION

Myiasis is the infestation of live humans and other vertebrate animals with dipterous larvae, which feed on the host's dead or living tissue. Clinically, myiasis may be classified as cutaneous, atrial, wound, intestinal, or urinary, depending on the location of the fly larvae (James and Harwood, 1969).

Intestinal myiasis in humans is probably an accidental myiasis related to ingestion of contaminated food or water containing fly larvae. Some larvae are destroyed by the digestive juice, but others are able to live in the intestinal tract and produce intestinal distress (Nagakura *et al.*, 1991).

Megaselia scalaris, the humped-back fly, is an insect of medical importance worldwide. In addition to causing myiasis in humans (Trape *et al.*, 1982, Singh et al. 1988, Singh & Rana 1989), it has also been reported as a forensically important fly (Barnes, 1990 and Disney,1994).

Previous records of this species as causative agent of myiasis in Egypt are not available.

The present work aimed at:(1) Determination of the role of *M.scalaris* as a cause of human intestinal myiasis (2) Confirmation of identification of *M.scalaris* larvae with the aid of (SEM) that provides the taxonomic information of this species, which might be useful to differentiate from other closely related species.

MATERIALS and METHODS

Collection of larvae:

Stool sample with a queery maggot infestation was obtained from human intestinal myiatic case in Assiut University Hospital; Specimen was put in clean plastic cup and brought to the Department of Parasitology, Faculty of Medicine. Larvae were picked up from stools with the aid of small brush.

Laboratory breeding and mounting of the larvae:

Larvae of intestinal myiatic cases were reared on stool culture, the procedure was done according to Zohdy and Morsy (1982). Mounting of larvae was done according to Morsy *et al.* (1991).

Studying morphological features and life stages:

Identification was based on morphological characteristics of larvae, pupae and adult flies according to the keys given by (Brues and Melander,1932, Zumpt, 1965 and Disney,1989).

Scanning Electron Microscopy:

The procedure was performed according to method described by Hayat (1981). Larvae were sent to the Electron microscope unit to be examined by scanning electron microscopy JEOL – JSM-5400 Lv and photographed.

RESULTS

On examination of stools, large numbers of small creamy white larvae were detected. After three days from keeping the larvae in the stool, the third stage larvae started to creep on the sidewall of the beaker until it became almost at its upper border for pupation. Some of them were collected, examined and mounted. The puparial stage took about 2-3 days to be completed. Adults started to emerge 4-5 days later. Most of the adults were collected and examined. After 1-2 days from the emergence of adults, numerous minute whitish banana-shaped eggs were seen floating on the surface of the stool. These eggs took about 2-3 days to be developed to the larvae. The whole cycle till the emergence of adults was 11-13 days.

Morphological features and life stages:

Identification as *Megaselia scalaris* (Phoridae, Diptera) was based on morphological characteristics of larvae, pupae and adult flies according to the keys given by (Brues *et al.*, 1932 Zumpt 1965, and Disney1989) as follows: -

1) **The larva:** (Plate I)

The third stage larva is about 4 mm long .It is creamy white in colour with the body pointed anteriorly and widens posteriorly. It is segmented into 12 segments bearing short fleshy processes. Cephalopharyngeal skeleton showed a pair of anteriorly curved mouth hooks. The anterior end of each mouth hook has seven finger like processes arranged in a fan-shaped manner. Just behind each mouth hook, there is an elongated hypostomal sclerite. The pharngeal sclerite has two ventral and two dorsal horns or arms. The ventral horns are more or less triangular in shape with pointed posterior end. The dorsal horns are wider than the ventral ones and nearly rectangular in shape. Both the ventral and dorsal horns are sickle-shaped (Plate I. Figs. 1 & 2). There are two longitudinal tubes extending from the anterior end (2^{nd} thoracic segment) to the posterior end. On the last abdominal segment, lie the posterior spiracles. On each segment, there is a sharp spine located bilaterally. Posterior spiracles are located on the last abdominal segment away from each other. The distance between them is nearly double the width of each plate. Each plate is formed of a widely opened C-shaped peritreme, which contains upper and lower apple shaped slits. Each one is clefted from its upper and lower sides. A spinous chitinous structure is found connecting these structures on the medial side (Plate I. Figs. 3 & 4).

2) **The pupa:** (Plate II)

It is brown in colour, 4x3mm, angular in shape with anterior narrow part, which has small 4 horn-like anteriorly projecting papillae. Pupae were found on the sidewall of the beaker near its upper margin away from stools, sometimes on the undersurface of the petri dish (Plate II Fig. 2).

3) Morphological features of the reared adult :

The whole cycle from oviposition of eggs till the emergence of adults was estimated to be 11-13 days. The adult fly is characterized by being small in size (2-4 mm. length). The thorax and abdomen are yellow to light brown with a variable dark brown to black pattern on the abdomen. In the male, the two bristles at the tip of the anal tube are long and feathery (Plate III. Fig. 1). In the female the ovipositor is prominent at the hind end of the abdomen (Plate III. Fig. 2). The legs are long and stoutly built with the hind femora having a characteristic dark spot at the apical ends which futher helps in the identification of *M scalaris* (Plate III. Fig. 2). Maxillary palpi consisting of one segment, antenna is dome

shaped, the 3rd segement bearing a dorsally placed bare arista (Plate III. Fig. 3). Wings (Plate III. Fig. 4) with anterior strong veins towards the anterior margins while others are oblique and weak, thorax strongly convex, hence the name humped back fly.

Scanning electron microscopy of third-instar of *Megaselia scalaris:*

SEM of *Megaselia scalaris* larvae confirmed the identification done by light microscope. The whole larva appeared with wrinkled intugment of 12 abdominal segments. The body is cylindrical and tapers toward the anterior end. There are bilateral spines on each segment extending from the third thoracic to the last abdominal segment. (Plate IV, Fig. 1). The cephalic segment: It is composed of a pair of antenna, a pair of maxillary palp complexes and a mouth part. The maxillary palp complex consists of several types of papillae. Mouth parts: The anterior part of the mouth revealed the presence of 2 lateral groups of 5-7 finger like processes each, arranged in a fan like manner. These 2 groups representing the anterior curved end of the mouth hooks (MH). The prominent features are the labium (L), labrum (LB), a pair of mouth hooks (MH) and an oral groove. A pair of sensillae is located ventrally at the end of the labrum. The labium is more or less a large triangular portion. Each mouth hook appears as a semi-circle lobe, with its rim composed of a 9 deep serrations (Plate IV, Fig. 2). The integument of the thoracic segments appeared to be smooth, while that of the whole abdominal segments appeared to be covered with dorsally and laterally short spinous processes (Plate IV, Fig. 3).

Magnification of the abdominal spine revealed that it is much thicker at its base and then progressively thinned out to be pointed at its end. A group of small sharp spines are found on the junction between thick and thin part and another group is found on the thin part. (Plate IV, Fig.4). Abdominal segments showed the presence of countless number of small processes covering the whole integument of these segments (plate IV. Fig. 5). Each process is formed of rounded anterior part (knob-like), which is inserted into a pit, and a free elongated pointed posterior part.

A large pointed spine or process projects from the lateral side of each segment Anterior spiracles are located on each latero-posterior edge of the prothorax. Each appears as oval-shaped; having two spiracular openings (slits), with one end closed together, while the other is far apart.

Posterior spiracles: A pair of cone shaped structures protruding dorsally on the 12th segment (Plate IV Fig 6 & 7.). Each appears as a

large and slender spiracular plate, which is constricted centrally. Each constriction is composed of two straight spiracular slits (S). The spiracular hairs (SH) appear centrally, at the area of constriction. (Plate IV. Fig. 8.)

DISCUSSION

Megaselia scalaris is a small fly with known scavenging properties (Zumpt, 1965).

The medical significance of *Megaselia scalaris* has been described previously in reports of larvae of this fly causing myiasis of wounds (Patton, 1922), eye (James, 1947) and urogenital system (Singh and Rana, 1989).

Megaselia scalaris was reported to cause intestinal myiasis by (Ingram, 1922; Rhodes-Jones , 1957andSingh *et al*., 1988)

Up till now there are no records of intestinal myiasis due to *Megaselia scalaris* in Egypt, as previous studies did not mention this fly while *Eristalis tenax* and *Sarcophaga* sp. were incriminated to be the cause intestinal myiatic cases reported by Khalifa and El-Gozamy (1995) and Khalifa and Mowafy (1997) respectively. Therefore, present work is the first record of human intestinal myiasis caused by *Megaselia scalaris* fly in Egypt.

In the present work, laboratory breeding of *Megaselia scalaris* larvae on stool media till emergence of the adults was done. It was found that stool medium was very suitable for the breeding of larvae, pupae and even the adult flies. This finding agreed with that of John Smart (1948).

Based on light microscopy; life stages (larva; pupa; and adult fly) of *Megaselia scalaris* were described in the present work. It was in agreement with the description of immature and adult stages of *M. scalaris* that have been previously described by (Zumpt 1965, Tumrasvin *et al*., 1977, Kaneko *et al*., 1978 Liu and Greenberg, 1989).

Several workers did SEM studies on different fly larvae. Khalifa and Mowafy (1997) studied *Sarcophaga* larva, Deflippis and Leite (1997) worked on *Dermatobia hominis* first instar larvae and Leite and Scott (1999) on second instar of *Gasterophilus nasalis* ,while Colwell and O'Connor (2000) unidentified sarcophagid maggots.

As regards scanning electron microscopy of *M. scalaris* it has been done to describe the egg only by Greenberg & Wells 1998.

In the present work, some details of the surface ultrastructure of the third-instar *M. scalaris* with the aid of SEM were described.

Particular attention was given to the morphology of the cephalic segment, anterior and posterior spiracles, in order to point out some taxonomic significance from the other *Megaselia* species.

Magnification of the abdominal spines revealed that this spine is much thicker at its base and then progressively thinned out to be pointed at its end. A group of small sharp spines are found on the junction between thick and thin parts and another group is found on the thin part. This characteristic has not been described before for this species.

According to the available literature, SEM of *M. scalaris* larva was done for the first time in Egypt. This might be explained by the fact that *Megaselia sp.* larvae usually passed unnoticed by the examiners due to their small size.

In conclusion: (1) *Megaselia scalaris* should be put into consideration as a causative agent of human intestinal myiasis in Egypt (2) Laboratory breeding of larvae was of great help in the identification of this fly and the description of the adult morphological features. (3) Data obtained by SEM on *Megaselia scalaris* larvae were very valuable and helpful for identification of such larvae.

The use of SEM for better identification of the unknown fly larvae is recommend. Moreover, we must be aware of the accidental ingestion *Megaselia scalris* fly eggs from contaminated food that leads to intestinal myiasis, which is a Potential health hazard.

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LIST OF FIGURES

(Plate I): ***Megaselia scalaris*** **larva.**

Fig. 1: Cephalo-pharngeal skeleton. (X200).

Fig .2: Mouth hooks. (X400).

Fig. 3: Posterior spiracles of the third stage larva. (X200).

Fig. 4: Magnifiation of posterior spiracles of the third stage larva. (X400).

(Plate II): ***Megaselia scalaris*** **pupa.**

Fig. 1: Pupa on the sidewall of the beaker near its upper margin away from stools, and on the undersurface of the petri dish.

Fig. 2: pupa. (X198).

(Plate III): ***Megaselia scalaris*** **adult stage.**

Fig. 1: Adult male. (X132). The arrow points to the two feathery bristles at the tip of the anal tube.

Fig. 2: Lateral view of adult female. (X132). The arrow points to the **ovipositor** at the hind end of the abdomen. Legs show that hind femora having a characteristic **dark spot** at the apical ends.

Fig .3: Antenna is dome shaped with bare arista (X80).

Fig. 4: *Megaselia scalaris* wing with characteristic strong anterior veins.

(Plate IV): **Scanning electron micrographs of the third-instar *Megaselia scalaris*.**

Fig. 1: ventral view showing wrinkled segments .The larva tapers towards the anterior end (AE). Bar= 500μm.

Fig. 2: cephalic segment illustrating the antenna, with mouthpart showing mouth hooks (MH) with a deep serrated rim, labrum (LB), and labium (L). Bar= 10μm.

Fig. 3: Lateral side of the abdominal segments showing the laterally protruded large spines and the minute processes covering each segment. Bar= 100μm.

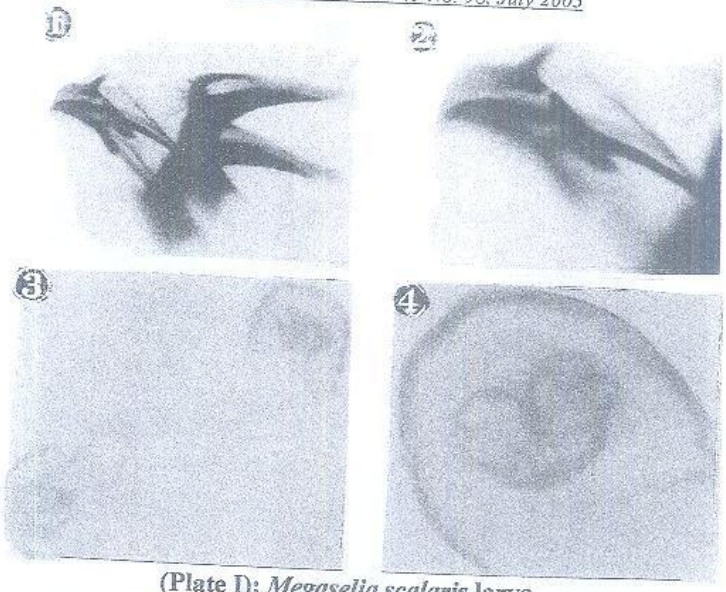
Fig. 4: higher magnification of the laterally protruded large spine. Bar=10μm.

Fig. 5: Abdominal segments with minute processes covering the surface of each segment. Bar= 50μm.

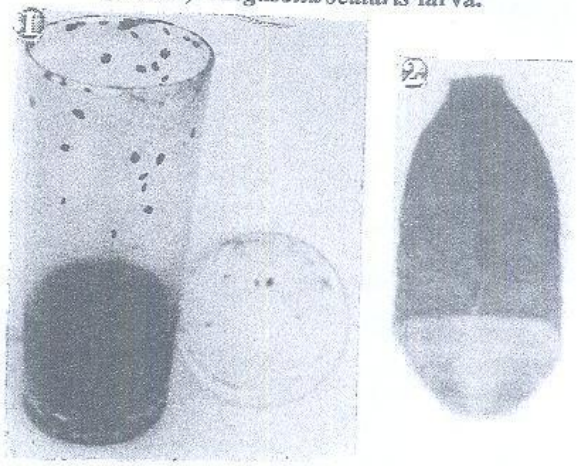
Fig. 6: postero-sagittal view showing a pair of protruding posterior spiracles (PS). Bar = 100μm.

Fig.7: Higher magnification of posterior spiracles. Bar=50μm.

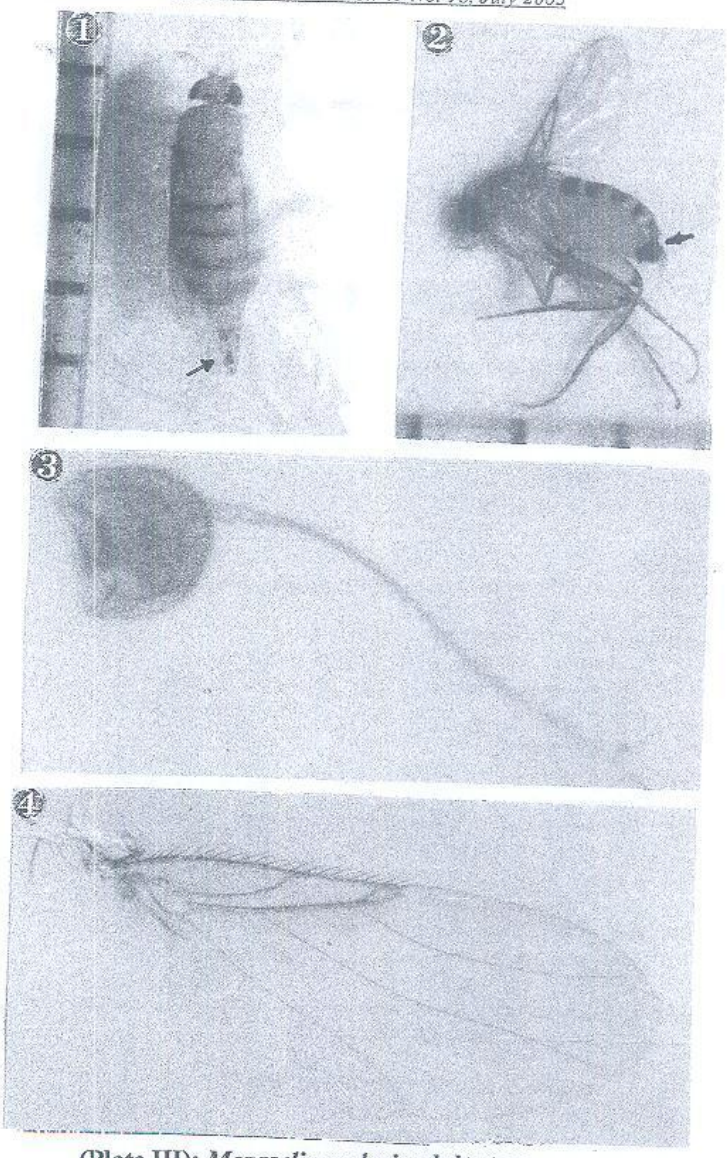
Fig8: Apical view of the posterior spiracle illustrating a slender spiracular disc that is constricted centrally. The arrows point to two straight slits located on each side of its constriction. Spiracular hair (SH) that appears in the center of the posterior spiracle. Bar=10μm.



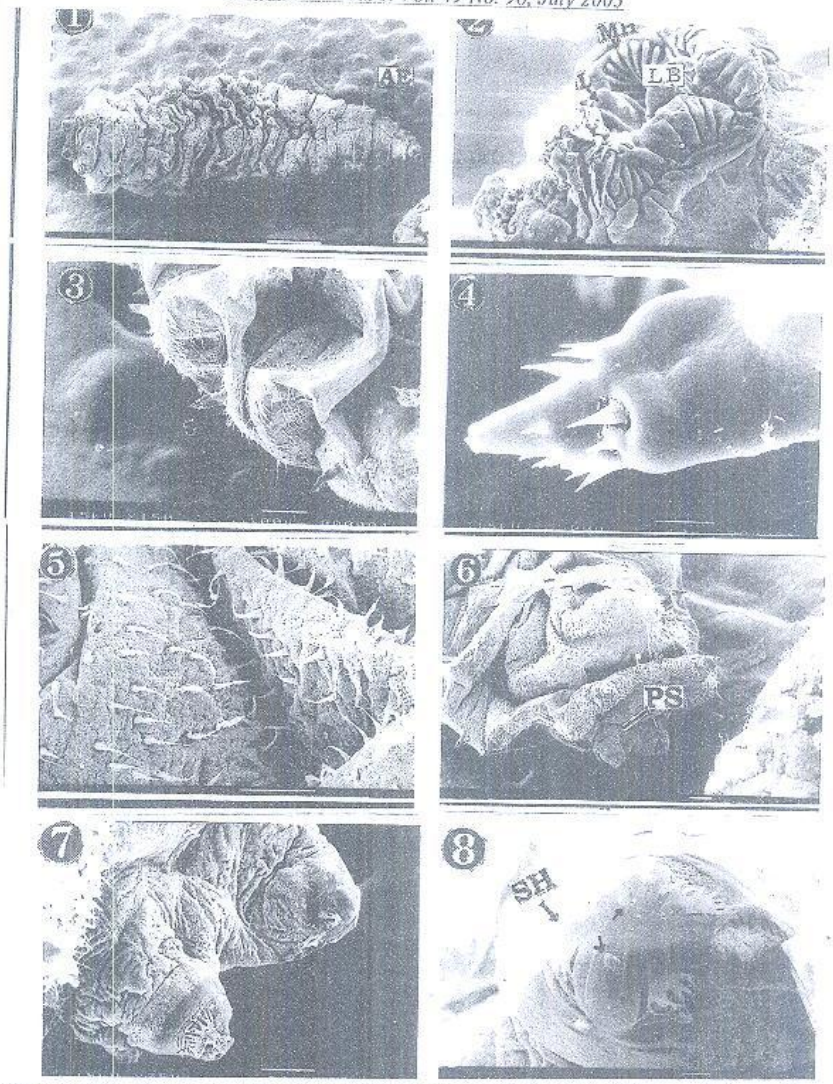
(Plate I): *Megaselia scalaris* larva.



(Plate II): *Megaselia scalaris* pupa.



(Plate III): *Megaselia scalaris* adult stage.



(Plate IV): Scanning electron micrographs of the third-instar *Megaselia scalaris*.