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A new Report of Parasitic Nematodes (Thelastomatidae) in Egyptian Cockroaches, *Polyphaga aegyptiaca* (Dictyoptera: Polyphagidae) in Sharkia Governorate, Egypt.

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ABSTRACT

There are many reports about thelastomatid parasitic nematode isolated from different species of cockroaches in many countries, without any clear information about those nematodes that are associated with *Polyphaga aegyptiaca* until now, so we have examined the nematodes were recovered from *P. aegyptiaca* and collected from Sharkia Gov., Egypt. *Hammerschmidtella diesingi* and *Leidynema appendiculata* were found with the high infection rates in *P. aegyptiaca*. The results indicated that the prevalence of cockroaches infected with *H. diesingi* alone or integrated with another species was (51%), similarly, *L. appendiculata* was (65%) from total cockroaches. The mean intensity of *L. appendiculata* was higher than *H. diesingi* in the current cockroaches. The gravid females of both *H. diesingi* and *L. appendiculata* were also higher than of any other stage of nematodes. Finally, using light and electron microscope, in order to identify and determine the morphological characteristics of both species of nematode. Results also indicated that SEM is a powerful tool to identify the morphological characteristics such as head, female cephalic region, vulva, tail, anus, male cloaca area and papillae.

INTRODUCTION

There are currently around 4,500 species of cockroaches on the globe, of which only are associated or adapted to environments transformed by humans Beccaloni & Eggleton (2011). Several studies have been carried out on the desert cockroaches belonging to the subfamily (Polyphagidae), especially the larger species of *Polyphaga* inhabiting cavities. *Polyphaga aegyptiaca* (L.) is one of the most widely distributed of these large species Grandcolas (1994). It is an uncommon cockroach that lives in desert or semi-desert environments and spread in African and Asian countries Beccaloni & Eggleton (2013). *P. aegyptiaca* commonly called the Egyptian desert roach and was observed in rock shelters in desert locations, each cavity harbored a small population. These groups generally included a few adults with larvae. Cockroaches burrowed in the sand at the darker parts of the rock shelters near the foot of the walls, where temperatures are lower. Oothecae were found in the sand and they were made up to 12 eggs Grandcolas (1996).

Thelastomatoidea is nematodes that are parasitic of invertebrates essentially arthropods Shah (2007). They live in cockroach hindgut and feed upon the host's gut contents like its bacterial microfauna and body fluid Jex *et al.* (2005). Nematodes belonging to the family Thelastomatidae have been reported more than forty species Ozawa *et al.* (2014).

The most frequent species were reported: *Hammerschmidtella diesingi* and *Leidynema appendiculatum* Ozawa *et al.* (2016). There is no report about parasitic nematodes recovered from *P. aegyptiaca* in Egypt, although they have been found in many species of cockroaches in other countries, mainly the *Periplaneta americana*, *P. fuliginosa*, and *Blattella germanica* have extremely high environmental adaptability and are now spreading and inhabiting around the world Appel & Smith (2002); Bell *et al.* (2007).

Infection prevalence of cockroaches with two common species of thelastomatoid nematodes, individually or combined, led to examine the internal structure of the cockroaches infected with any species of them Jex *et al.* (2005). Because there is no report about associated nematodes in the Egyptian cockroach, we surveyed *P. aegyptiaca* in Sharkia governorate, Egypt for its nematode associates. The present study aimed to examine the prevalence, intensity and morphological characterization of two species of nematodes *H. diesingi* and *L. appendiculata* recovered from the host.

MATERIALS AND METHODS

Sample Collection:

A total of (49) adult *P. aegyptiaca* cockroaches were collected directly with forceps from old houses in the desert villages, near to Zagazig, Egypt. Cockroaches stored in plastic cages, under laboratory conditions until dissection, at the laboratory of invertebrate, Zoology Department Faculty of Science, Zagazig University.

Dissection of the Cockroach and Nematode Preservation:

Each cockroach was preserved in a sterile tube containing cotton soaked in diluted 10% chloroform then transported to the laboratory for parasitological analyses. Roaches were anesthetized with cold at 5°C for 3 minutes and then dissected under a microscopic stethoscope. The hindgut was

separated from the digestive tract. The nematodes were found alive in the hindgut of 38 roaches were collected by a micropipette with a baster tube into a small vial. For their taxonomic and morphological studies, they were killed in distilled water at 60°C for 3 minutes. Nematodes were first fixed in 70% ethanol, preserved in a solution of 5% glycerin and 95% of 70% ethanol for clearing. These were left a Petri dish half-covered at room temperature for 48 hours, to allow the ethanol to evaporate, thereby leaving nematodes in glycerin. The nematodes were individually installed on glass slides and photographed by using Olympus research photomicroscopy and measured with an optical micrometer. All measurements are in millimeters (mm).

Specimen Preparation for SEM Observation:

Nematodes were transferred in primary fixed with 2.5% glutaraldehyde + 2% formaldehyde, and washed 3 x 15 min. in 0.1 M sodium phosphate buffer pH 7.4 + 0.1 M Sucrose, post-fixed with 2% sodium phosphate buffered osmium tetroxide pH 7.4, and washed 3 x 15 min in 0.1 M sodium phosphate buffer pH 7.4, dehydrated sequentially with ethanol (50%, 80%, 90%, 96%, 100% / 2 x 15 min each), contrasted overnight using 70 % acetone + 0.5 % uranyl acetate + 1% phosphotungstic acid, after dehydrated with ethanol (in distilled water), at 4° C. Nematodes were then coated with gold-palladium membranes and observed in a Jeol JSM-6510 L.V SEM. The microscope was operated at 30 KV at EM Unit, Mansoura University, Egypt.

Statistical Analysis:

Infection prevalence was calculated as a percent of infected cockroaches of the total sample. Mean intensity was calculated as an average number of nematodes per infected cockroach and standard deviation. Morphometric measurements were used the mean and range of data. Descriptive data were analyzed with SPSS 19.0.

RESULTS

Results in a Table (1) showed that the number of adult females, adult male and nymph of *P. aegyptiaca* cockroaches were dissected and infected with *H. diesingi* and *L. appendiculata*, only or both species of nematodes. A total of (49) Cockroach

dissected, (38) Cockroach infected by the nematode, (6) Cockroach infected by only *H. diesingi*, (13) Cockroach infected by only *L. appendiculata* and (19) Cockroach infected by both species of nematodes. The percentage of infection is (77.6%, 12.24%, 26.53%, and 38.78%, respectively).

Table (1): Number of cockroaches dissected and infected with one or both species of nematodes, in *P. aegyptiaca* cockroaches.

| Observations | No. of Cockroaches | | | | (% of Roaches |
|-------------------------------------|--------------------|---------|-------|-------|---------------|
| | Adult ♀ | Adult ♂ | Nymph | Total | |
| Dissected cockroaches | 21 | 12 | 16 | 49 | 100 |
| Infected cockroaches | 19 | 8 | 11 | 38 | 77.6 |
| H. diesingi only | 3 | 1 | 2 | 6 | 12.24 |
| L. appendiculata only | 6 | 3 | 4 | 13 | 26.53 |
| Both species of nematodes | 10 | 4 | 5 | 19 | 38.78 |
| Max. infected by H. diesingi | 13 | 5 | 7 | 25 | 51.02 |
| Max. infected by L. append. | 16 | 7 | 9 | 32 | 65.30 |

* Maximum Cockroaches infected by *H. diesingi* = (*H. diesingi* only + both species of nematodes), and also Maximum Cockroaches infected by *L. appendiculata*.

Results in the Table (2) indicated that the number of *H. diesingi* and *L. appendiculata* in different stages were recovered from *P. aegyptiaca* cockroaches. The number of *L. appendiculata* was higher than the number of *H. diesingi*, the gravid females of both *H. diesingi* and *L.*

appendiculata were also higher than any other stage of nematode (36 and 48, respectively). Results in the same table presented superiority of the cockroaches infected with *L. appendiculata* in current sexes and stage of the nematode.

Table (2): Number of nematodes recovered from infecting cockroaches and percentage of infection prevalence, in *P. aegyptiaca* cockroaches.

| Examinations Nematode stage | nematodes recovered (No.) | | Cockroaches infected (No.) | | Infection prevalence (%) | |
|--------------------------------|------------------------------|-----|-------------------------------|----|-----------------------------|-------|
| | H. | L. | H. | L. | H. | L. |
| Adult Male | 4 | 9 | 2 | 4 | 4.08 | 8.16 |
| Adult Female | 17 | 28 | 9 | 10 | 18.37 | 20.41 |
| Gravid Female | 36 | 48 | 11 | 12 | 22.49 | 24.48 |
| Juvenile | 6 | 15 | 3 | 6 | 6.12 | 12.24 |
| Total | 63 | 100 | 25 | 32 | 51.02 | 65.30 |

Finally, results in Table (2) cleared the percentage of infection prevalence of both nematodes, prevalence percentages of *H. diesingi* were (4.08%, 18.37%, 22.49%, and 6.12%) respectively in current stages of nematode, percentages prevalence of *L. appendiculata* were (8.16%, 20.41%, 24.48%, and 12.24%, respectively) in current sexes and stage of nematode. So that infection prevalence by *L. appendiculata* in *P. aegyptiaca* cockroaches higher than *H. diesingi*.

Data in Figure (1) also cleared the total number of both species nematode recovered from the total number of cockroaches, and the infection prevalence of both nematodes, *H. diesingi*. And *L. appendiculata* were (51% and 65%, respectively).

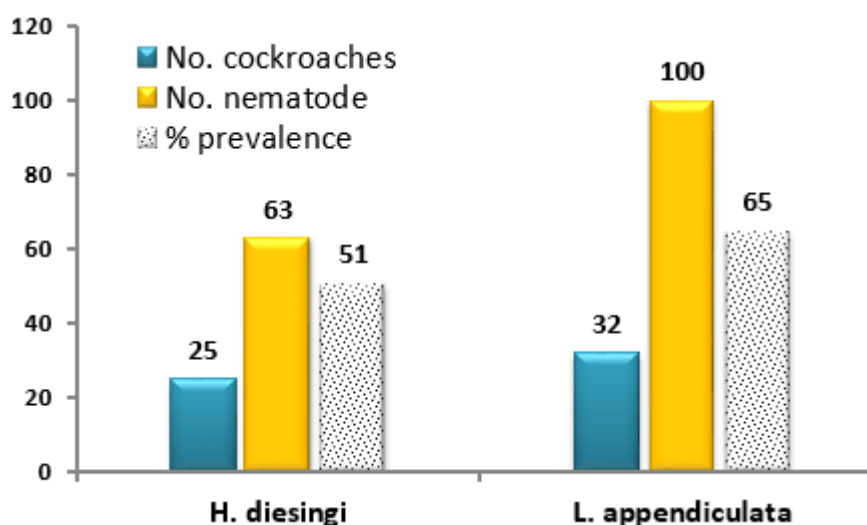


Fig. (1). distribution of nematodes in infected cockroaches and percentage of infection prevalence, in *P. aegyptiaca* cockroaches.

Data summarized in Table (3) that mean intensity of both nematodes recovered from *P. aegyptiaca* in current sexes and stage. Intensity of *L. appendiculata* was higher than *H. diesingi* in adult female, adult male and nymph cockroaches, (3.71, 2.21, and 2.15, respectively) at *L. appendiculata*,

(2.21, 1.45, and 1.75, respectively) at *H. diesingi*. The gravid females of both nematodes were also higher than of any other stage. (2.75, 2.50, and 3.33, respectively) at *H. diesingi*, (4.33, 3.25, and 2.65, respectively) at *L. appendiculata*.

Table (3): Mean intensity of nematodes *H. diesingi* and *L. appendiculata* recovered from *P. aegyptiaca*, according to host stage.

| Nematode stages \ Roaches stages | Intensity of nematodes | | |
|----------------------------------|---------------------------------|-------------------------------|---------------------------|
| | Adult female (mean \pm SD) | Adult male (mean \pm SD) | Nymph* (mean \pm SD) |
| <i>H. diesingi</i> | 2.21 \pm 1.10 | 1.45 \pm 0.77 | 1.75 \pm 0.82 |
| Adult Male | 2.00 \pm 0.00 | 0.00 \pm 0.00 | 1.00 \pm 0.00 |
| Adult Female | 2.05 \pm 0.96 | 1.33 \pm 0.58 | 1.50 \pm 0.70 |
| Gravid Female | 2.75 \pm 1.22 | 2.50 \pm 0.70 | 3.33 \pm 1.25 |
| Juvenile* | 2.00 \pm 1.41 | 0.00 \pm 0.00 | 2.00 \pm 0.00 |
| <i>L. appendiculata</i> | 3.71 \pm 1.35 | 2.21 \pm 0.85 | 2.15 \pm 1.11 |
| Adult Male | 3.00 \pm 1.41 | 1.00 \pm 0.00 | 2.00 \pm 0.00 |
| Adult Female | 3.25 \pm 0.75 | 2.67 \pm 1.15 | 2.50 \pm 0.71 |
| Gravid Female | 4.33 \pm 1.50 | 3.25 \pm 0.75 | 2.65 \pm 1.15 |
| Juvenile* | 3.50 \pm 0.70 | 1.00 \pm 0.00 | 2.33 \pm 0.57 |

* It is difficult to distinguish the sex of cockroaches and nematodes in the Nymph and juvenile stage, respectively

Description of *H. diesingi*

Data in Table (4) and Plate (1) cleared that morphometric characters of adult *H. diesingi* nematode recovered from *P. aegyptiaca*, according to host sexes.

Female: (figures 1-5); A spindle rounded body with tapered ends, 2.98 mm long, a maximum width of 0.25 mm, recorded in vulva region. Narrow lateral alae extended between posterior esophagus to the anus. Cuticle is deeply annulated up to the

base of the pseudobulb with simply striated throughout the body. Cephalic extremity formed by two annules, head and first annule. Labial papillae surrounded the mouth with labial ring by 0.024 mm wide. Amphids small and pore shaped, surrounded by slightly elevated cuticular rings. The buccal cavity is 0.011 mm long and 0.012 mm wide. Oesophagus 0.327 mm in total length, the corpus divided into two parts, an anterior part 0.115 mm long by 0.028 mm wide, a posterior part represented by a large pseudo bulb 0.092 mm long by 0.059 mm wide. Isthmus 0.042 mm long by 0.025 mm wide, surrounded by nerve ring, followed by basal bulb 0.092 mm long by 0.116 mm wide. Nerve ring located at 0.105 mm from the anterior end of the body, in the cylindrical portion of corpus, near the beginning of pseudobulb. Esophageal- Intestinal valve situated at 0.335 mm from anterior end. Excretory pore appeared slightly behind of Esophageal- Intestinal valve, at 0.32 mm from the anterior end of the body. Vulva a ventromedial transverse slit in the alignment of oesophagus base, located at 0.633 mm from anterior end, vagina and uterus posteriorly directed. Uterus divides into two uteri; each uterus connected to an oviduct, uterus filled with several oval eggs, 0.077 long by 0.033 wide. Anus situated at 0.074 mm from the tip of the tail. The tail is very long and thin measuring 0.914 mm long.

Male: (figures 6-8); A slimmer body with a thin cuticle, measuring 0.795 mm

long, cephalic and posterior end truncated, curved at the posterior end, 0.061 mm maximum width at the level of oesophagus base. Lateral alae absent. Cephalic extremity formed by single expanded annule, Cuticle annulated; gradually widening from narrow annules at the anterior region and isthmus level to maximum width annulus at oesophagus base level, annule size is maintained along the remainder body. Labial papillae and amphids are very small compared with female. The buccal cavity is short and narrow than the female by 0.004 mm long and 0.006 mm wide. Muscular oesophagus 0.131 mm in total length, moderately corpus 0.044 mm long by 0.010 mm wide, isthmus 0.032 mm long by 0.011 mm wide, and basal bulb less rounded and developed than in female 0.030 mm long by 0.020 mm wide. Nerve ring located at the middle of the isthmus at 0.089 mm away from anterior end. Oesophageal- Intestinal valve and excretory pore located (0.134 and 0.161 mm, respectively), from the anterior end of the body. Anterior region of Intestine is the maximum width and posterior region dilated near the junction with testis. One testis of large size, extended from the cloaca opening to mid-body, single spicule by 0.028 mm in length. Four pairs of papillae, arranged in preanal, postanal papillae and caudal appendage base. Anus situated at 0.026 mm from the tip of the tail. The tail is smaller than female, 0.105 mm long.

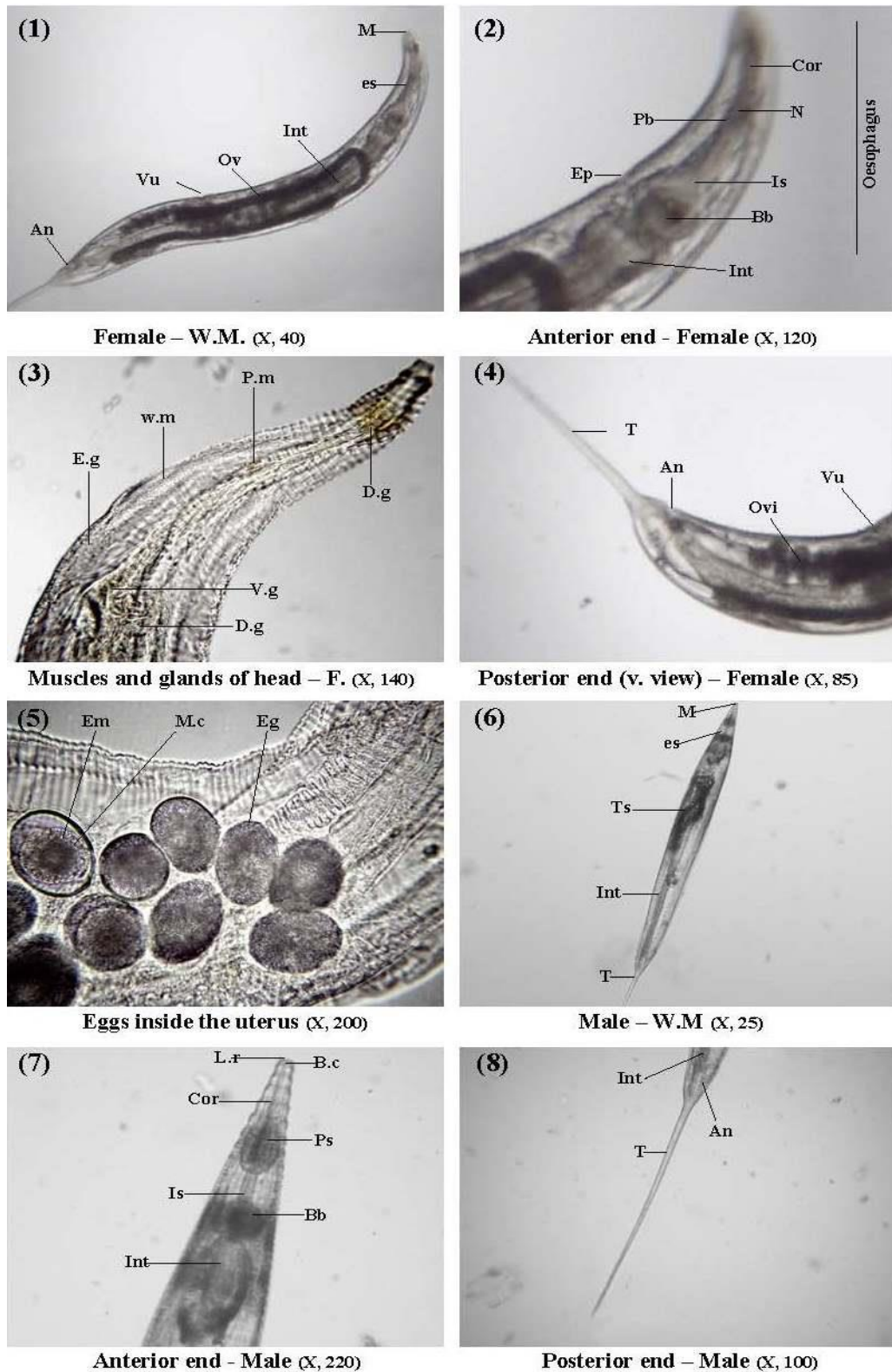


Plate (1). Photomicrographic details of adult *H. diesingi* nematode recovered from *P. aegyptiaca*, according to host sexes, showing Pseudobulb (Ps), Corpus (cor), Nerve ring (N), Excretory pore (Ep), Isthmus (Is), Basalbulb (Bb), Intestine (Int), Mouth (M), Oesophagus (es), Testis (Ts), Ovary (Ov), Vulva (Vu), Anus (An), Tail (T), Oviduct (Ovi), Pharyngeal muscles (P.m), wall musculature (w.m), Excretory glands (E.g), Dorsal gland (D.g), Ventral gland (V.g), Embryo (Em), Many-cells (M.c), Egg (Eg), Labial ring (L.r), Buccal cavity (B.c).

Table (4): Morphometric characters of adult *H. diesingi* nematode recovered from *P. aegyptiaca*, according to host sexes.

| Measurements | Nematode sexes | |
|--------------------------------|--------------------------------|------------------------------|
| | Adult female (mean - Range) | Adult male (mean - Range) |
| No. | 53 | 4 |
| Body length | 2.984 (2.175-3.389) | 0.795 (0.516-0.941) |
| Body width | 0.251 (0.169-0.292) | 0.061 (0.046-0.089) |
| Buccal cavity length | 0.011 (0.008-0.013) | 0.004 (0.003-0.006) |
| Buccal cavity width | 0.012 (0.009-0.014) | 0.006 (0.005-0.009) |
| Labial ring width | 0.024 (0.021-0.028) | --- |
| Oesophagus length | 0.327 (0.279-0.381) | 0.130 (0.109-0.147) |
| Corpus length | 0.115 (0.087-0.151) | 0.044 (0.041-0.049) |
| Corpus width | 0.028 (0.023-0.033) | 0.010 (0.009-0.013) |
| Pseudobulb - anterior end | 0.191 (0.162-0.218) | 0.065 (0.056-0.073) |
| Pseudobulb length | 0.092 (0.079-0.098) | 0.017 (0.015-0.021) |
| Pseudobulb width | 0.059 (0.039-0.069) | 0.014 (0.012-0.018) |
| Isthmus length | 0.042 (0.038-0.049) | 0.032 (0.024-0.038) |
| Isthmus width | 0.025 (0.018-0.031) | 0.011 (0.008-0.015) |
| Basal bulb length | 0.075 (0.066-0.079) | 0.030 (0.024-0.034) |
| Basal bulb width | 0.077 (0.065-0.088) | 0.020 (0.014-0.027) |
| Nerve ring - anterior end | 0.105 (0.079-0.124) | 0.089 (0.071-0.095) |
| Intestine valve - anterior end | 0.335 (0.284-0.389) | 0.134 (0.115-0.156) |
| Excretory pore - anterior end | 0.321 (0.291-0.404) | 0.161 (0.131-0.215) |
| Vulva - anterior end | 0.633 (0.467-0.746) | --- |
| Anus width | 0.074 (0.055-0.083) | 0.026 (0.021-0.032) |
| Spicule length | --- | 0.028 (0.023-0.037) |
| No. of papillae | --- | 4.000 (4.000-4.000) |
| Tail length | 0.914 (0.715-1.088) | 0.105 (0.089-0.125) |
| Egg length | 0.077 (0.065-0.083) | --- |
| Egg width | 0.033 (0.030-0.039) | --- |

Description of *L. appendiculata*

Data in Table (5) and Plate (2) cleared that morphometric characters of adult *L. appendiculata* nematode recovered from *P. aegyptiaca*, according to host sexes.

Female: (figures 1-6); Body cylindrical, decreasing at the anterior and posterior end, 2.833 mm long, maximum width of 0.275 mm, recorded in gonad region of the body. Lateral alae also emerged near the vulva and extended down at the posterior end. Cephalic extremity formed by two annulus, cuticles annulated throughout the length of the body. Labial papillae surrounded the mouth with labial ring by 0.022 mm wide. Buccal cavity is 0.012 mm long and 0.015 mm wide. Oesophagus 0.480 mm in total length, subdivided into many parts, the corpus divided into narrow

anterior part 0.274 mm long by 0.025 mm wide, and pseudobulb 0.147 mm long by 0.041 mm wide, isthmus 0.023 mm long by 0.031 mm wide, and basal bulb 0.092 mm long by 0.116 mm wide. Nerve ring located at 0.133 mm from anterior end of the body. Oesophageal- intestinal valve situated at 0.509 mm from anterior end. Excretory pore appeared slightly behind of oesophageal-intestinal valve, at 0.487 mm from the anterior end of the body. The anterior part of intestine enlarged with blind diverticulum but posteriorly is cylindrical. Vulva transversely located near the middle of the body, at 1.581 mm from anterior end. Anus situated at 0.044 mm from the tip of the tail. Two ovaries, uterus filled with several oval eggs and flattened slightly on one side, 0.073 long by 0.042 wide. The tail is short and filiform measuring 0.298 mm long.

Male: (figures 7, 8); Body is a smaller than female, curved at the posterior end, measuring 0.885 mm long, 0.117 mm maximum width, recorded in the posterior esophagus region of the body. Lateral alae also presented and extend throughout the length of the body. Cephalic extremity formed by single expanded annulus, cuticles annulated throughout the length of the body and striated up to the posterior corpus from the head end. Labial papillae surround the mouth with labial ring by 0.022 mm width. The buccal cavity was 0.014 mm long and 0.022 mm wide. Oesophagus 0.107 mm in total length, corpus 0.067 mm long by 0.015

mm wide, it has a uniform diameter with unclear pseudobulb, isthmus 0.007 mm long by 0.014 mm wide, and basalbulb 0.032 mm long by 0.027 mm wide. Nerve ring, oesophageal- intestinal valve and excretory pore located at (0.122 and 0.132 mm, respectively) from anterior end of the body. Intestine appeared without any diverticulum or loop. Testis enlarged, occupying two posterior ends of the body. Anus situated at 0.024 mm from the tip of the tail. The tail is smaller than female, 0.016 mm long, provided with (3-5) pairs of large papillae. One spicule was 0.028 mm in length.

Table (5): Morphometric characters of adult *L. appendiculata* nematode recovered from *P. agyptiaca*, according to host sexes.

| Measurements | Nematode sexes | |
|--------------------------------|--------------------------------|------------------------------|
| | Adult female (mean - Range) | Adult male (mean - Range) |
| No. | 76 | 9 |
| Body length | 2.833 (2.550-3.100) | 0.885 (0.875-0.905) |
| Body width | 0.275 (0.230-0.290) | 0.117 (0.112-0.128) |
| Buccal cavity length | 0.012 (0.011-0.013) | 0.015 (0.014-0.017) |
| Buccal cavity width | 0.015 (0.014-0.017) | 0.014 (0.013-0.015) |
| Labial ring width | 0.022 (0.019-0.027) | 0.022 (0.021-0.025) |
| Oesophagus length | 0.480 (0.465-0.510) | 0.107 (0.105-0.110) |
| Corpus length | 0.274 (0.260-0.301) | 0.067 (0.063-0.075) |
| Corpus width | 0.025 (0.020-0.035) | 0.015 (0.015-0.016) |
| Pseudobulb - anterior end | 0.289 (0.278-0.310) | --- |
| Pseudobulb length | 0.147 (0.145-0.152) | --- |
| Pseudobulb width | 0.041 (0.039-0.045) | --- |
| Isthmus length | 0.023 (0.022-0.025) | 0.007 (0.007-0.008) |
| Isthmus width | 0.031 (0.030-0.033) | 0.014 (0.013-0.015) |
| Basal bulb length | 0.092 (0.085-0.105) | 0.032 (0.031-0.035) |
| Basal bulb width | 0.116 (0.110-0.127) | 0.027 (0.026-0.030) |
| Nerve ring - anterior end | 0.133 (0.125-0.150) | 0.071 (0.067-0.074) |
| Intestine valve - anterior end | 0.509 (0.497-0.522) | 0.122 (0.118-0.125) |
| Excretory pore - anterior end | 0.487 (0.482-0.495) | 0.132 (0.130-0.136) |
| Vulva - anterior end | 1.581 (1.373-1.756) | --- |
| Anus - tip of tail | 0.459 (0.451-0.475) | 0.027 (0.026-0.029) |
| Anus width | 0.044 (0.039-0.046) | 0.024 (0.022-0.025) |
| Spicule length | --- | 0.028 (0.027-0.030) |
| No. of papillae | --- | 3.667 (3.000-5.000) |
| Tail length | 0.298 (0.294-0.310) | 0.016 (0.015-0.018) |
| Egg length | 0.073 (0.070-0.078) | --- |
| Egg width | 0.042 (0.041-0.045) | --- |

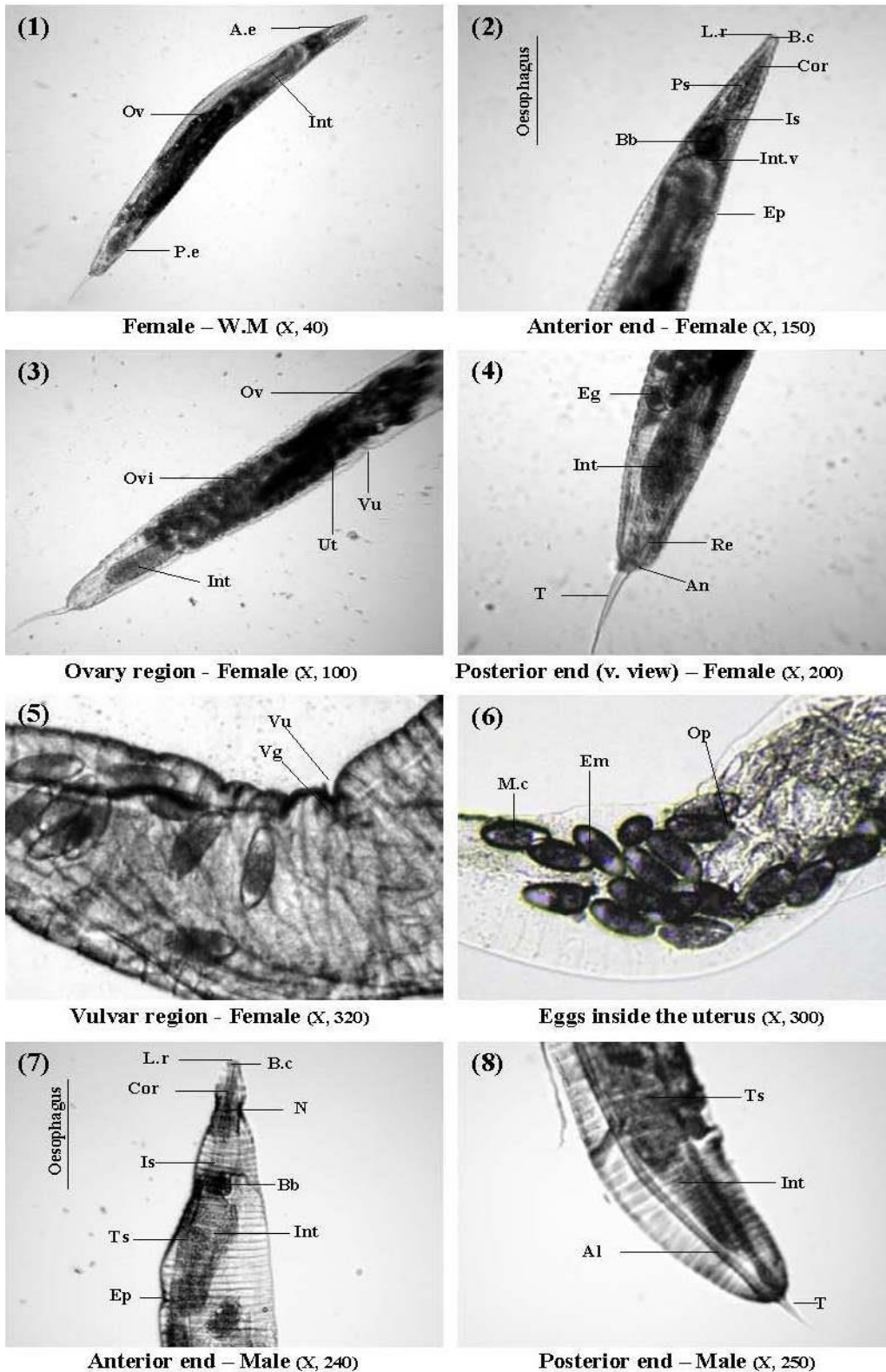


Plate (2). Photomicrographic details of adult *L. appendiculata* nematode recovered from *P. aegyptiaca*, according to host sexes, showing Anterior end (A.e), Ovary (Ov), Intestine (Int), Posterior end (P.e), Labial ring (L.r), Buccal cavity (B.c), Pseudobulb (Ps), Corpus (cor), Basalbulb (Bb), Isthmus (Is), Intestine valve (Int.v), Excretory pore (Ep), Ovary (Ov), Oviduct (Ovi), Vulva (Vu), Uterus (Ut), Egg (Eg), Rectum (Re), Anus (An), Tail (T), Vagina (Vg), Many-cells (M.c), Embryo (Em), Operculum (Op), Testis (Ts), Alae (Al).

SEM of *L. appendiculata* (Plate 3). Adult female (figures 1- 6); 1. Habitus of the adult. 2. Front lateral view of Cephalic region with anterior annule. 3. Lateral view of the anterior end with labial ring. 4. En face view of cephalic region with amphidial sensory pore. 5. Ventral view of Vulva

region and excretory pore. 6. Ventral view of the tail region with anus and phasmid sensory pore. Adult male (figures7, 8); 7. Lateral view of cephalic region with anterior annule. 8. Lateral view of the posterior end with tail, alae, anus and papillae.

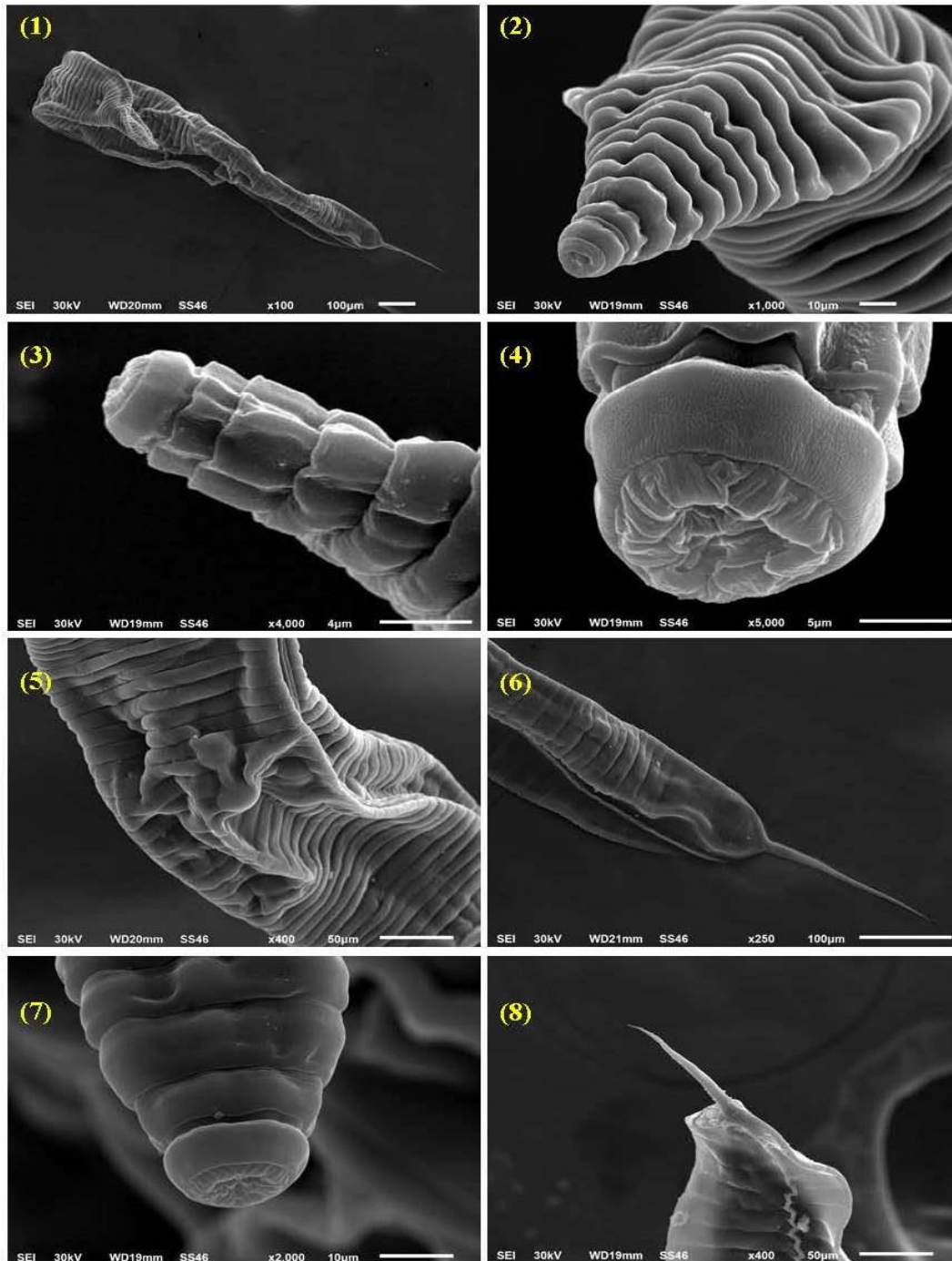


Plate. (3). Scanning electron microscopy of adult *L. appendiculata* nematode recovered from *P. aegyptiaca*, according to host sexes.

DISCUSSION

Dissection results of 38 cockroaches indicated that they were infected with two types of nematodes; *H. diesingi*. and *L. appendiculata*. This is the new report of a thelastomatid nematode isolated from Egyptian cockroaches, *Polyphaga aegyptiaca* (Dictyoptera: Polyphagidae) in Egypt.

Nematode had the potential for extensive infection and had a strong link with the cockroach species that spread all over the world Nakano (2013). Infection with multiple species of thelastomatid nematodes is common in cockroaches, where at least 15 species of unknown nematodes have been isolated from the Japanese wood cockroaches, and two known nematodes of American cockroaches Jex *et al.* (2005); Jex *et al.* (2006). Numbers of adult male nematodes were usually fixed to one, while adult the female was a few in female cockroaches. Juveniles were always present in any host age in the cockroach hindgut. The mechanism of the regulations not clear, but is hypothesized to be regulated by the nematode itself Zervos (1988). Nematodes were found in the front part of the hindgut behind to the pyloric valve, in a single or combined case, in the form of larvae or adults for both *H. diesingi* and *L. appendiculata*. Cockroach hindgut harbored with nematodes more than other parts of the gut (84%) of the total nematodes Alex & Minabelema (2013). The infection of nematode was higher in adult female cockroaches than any other stage, particular, the gravid females of both *H. diesingi* and *L. appendiculata*. The infection of cockroaches in *H. diesingi* was greater than that of *L. appendiculata* in *P. Americana* Verna Holoman, B. S. (1980). On the contrary, the results of the current study showed clear superiority of the *L. appendiculata* in the percentage of infection in *P. aegyptiaca* compared to *H. diesingi*.

The high-intensity cockroaches led to an easy infection of both *H. diesingi* and *L. appendiculata*, previous studies indicated

that the *P. fuliginosa* might be a more appropriate host for *L. appendiculata* than *P. Americana* Ozawa *et al.* (2014). Nematode prevalence was lower in *P. japonica* (65%,), *B. nipponica* (53%), and *P. surinamensis* (51%) when compared with that in *P. fuliginosa* (78%), *L. appendiculatum* successfully infected all three hosts Ozawa & Hasegawa (2017). Recently, surveys of parasitic nematodes in cockroaches were reported. *L. appendiculatum* was isolated from *P. americana*, *P. australasiae*, and *P. surinamensis* with a relatively low prevalence (14%, 31%, and 6%, respectively; Sinnott *et al.* (2015). *L. appendiculatum* was isolated from *P. fuliginosa* collected in Japan with high prevalence. Infection prevalence, intensity, and infra-population of *L. appendiculatum* in five host cockroach species were similar Ozawa *et al.* (2016). Prevalence of infection with three thelastomatid fauna of two species of cockroach ranged from 2 to 81% in *Panesthia cribrata* cockroach, and from 3 to 97% in *Panesthia tryoni* cockroach Jex *et al.* (2005).

The results of the infection prevalence of *H. diesingi* isolated from the gastrointestinal system of cockroaches (65%) were found among five other species of nematodes Hadi & Muhammed (2010). The results also indicated that cockroaches were infected with adult *H. diesingi* had the highest percentage of infection (46%) compared to other types of parasites Alex & Minabelema (2013). Infection prevalence of the larva and adult nematodes reached (4.8%, 0.08%, respectively), within the total (700) insect were collected and examined individually Thyssen *et al.* (2004).

The genus *Hammerschmidtella* was initially marked as amphidelphic Chitwood (1932), but in recent studies, they clearly described the reproductive system of *H. diesingi* as didelphic-prodelphic Shah (2007); Blanco *et al.* (2012). Some measurements of *H. diesingi* Females were recently found in cockroaches, such as buccal cavity length, isthmus length and

vulva from the anterior end were much higher than the values known for the species. The range obtained for the location of the nerve ring in males. The excretory pore at the base of the esophagus, agreed with previous descriptions Carreno and Tuhela (2011).

In the present study, we clearly observed the arrangement of papillae and agreed with descriptions of Shah (2007) and Blanco *et al.* (2012) showed that Papillae of *H. diesingi* consisted of four pairs, subventral pre-anal, adanal, subventral just posterior to anus, and duplex papilla at the base of the tail appendage Rina S. *et al.* (2016). Besides, Phasmids observed in only females. The gubernaculum is one of the controversial characteristics of *H. diesingi*, where many previous studies have pointed out its absence of this structure, located dorsally to spicule and bearing a ventral groove. Results of the current study agreed with most results of morphometric characters of females and males of *H. diesingi* detected in *P. americana* in Argentina Shah (2007); María *et al.* (2012); Nora & Villalobos (2012); Rina Sriwati *et al.* (2016).

The place where the insect lives is a considered a confined space where the cockroaches reproduce and are easily infected by the thelastomatid nematodes. This nonspecific niche superposition behavior as a specific survival mode must take into account that *Periplaneta* is paraphyletic Kambhampati, (1995). *L. appendiculata* might be able to re-infect easily when its hosts were reared at a high-density Stock (1988). Many species of the family Thelastomatidae were sympatric, because they were closely related evolutionarily, they had reproductive isolation, they showed an absence of competition for food in the digestive tract, and they had a morphologically similar, but well-differentiated Adamson & Noble (1993).

Results of studies indicated that cockroaches were infected with different types of nematodes is common, but only the

L. appendiculata was examined Jex *et al.* (2005); Jex *et al.* (2006). Reports showed that *L. appendiculata*, *H. diesingi* and other nematodes coexisting in the gut of cockroaches, without any evidence of competition between them Connor & Adamson (1998). A number of adult male *L. appendiculata* was approximately fixed but the adult female was mutable in the cockroaches gut. *L. appendiculata* juveniles were always present in any host age, because the egg supply and juvenile discharge may be continuously occurring to sustain an adult population in the cockroach hindgut Adamson & Noble (1993). Morphological and molecular characteristics of the thelastomatid nematode *L. appendiculata* from the smokybrown cockroach were examined in different host sexes and stages Ozawa *et al.* (2014).

This study has been agreed with several studies dealing with morphological characteristics of *L. appendiculata*, in terms of Mouth surrounded by large papillae and amphids. Lateral alae present. Oesophagus consisted of the corpus, pseudobulb, isthmus and basal bulb. The corpus in females consists of two cylindrical parts of which the posterior is wider than the anterior one. Excretory pore located at post-esophageal. Vulva near the middle of the body. Intestine may have a loop in the posterior part of the body. Eggs ellipsoidal. Caudal papillae three to five pairs. Spicule present Shah (2007); Sevdan *et al.* (2013); Sangeeta *et al.* (2014); Ozawa *et al.* (2014) and Sharaf *et al.* (2018).

Photomicrographic details of *L. appendiculata* adult female clearly showed Cephalic region by two annulus, first annule around the mouth and on which the lips were located, the second annule located between the first cephalic ring and the first somatic annule. Labial papillae surrounded the mouth with the labial ring. Xiong & John Crites (1986) mentioned that it is necessary to distinguish these two rings from the somatic annulus because they are morphologically different. Cuticle markedly annulated from the end of the first cephalic annule to the level of the anus. Lateral alae also emerged

near the vulva and extended down at the posterior end.

Our SEM figures of *L. appendiculata* adult female presented only amphids on the first cephalic ring by the lateral view of the anterior end. These results agreed with Trett & Lee (1981) mentioned that Phasmids are important characteristics of the class Secernentea but we were able to see them only in the female. Ventral view figures of excretory pore presented in both light and electronic microscopy. however, Xiong & John Crites (1986) founded that female excretory pore can only be seen with SEM and very little has been done with SEM in other closely related species one cannot say whether it is unique to this species or is common among other species. Ventral view figures of the vulva region showed that vulva transversely located near the middle of the body. SEM figures also presented that tail of adult male was smaller than female and provided with three up to five pairs of large papillae Praveen Kumar *et al.* (2014); Shah (2007); Anshu *et al.* (2011) and Rehana *et al.* (2016).

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