

## DESCRIPTION OF SOME NEMATODE PARASITES INFECTING AMERICAN COCKROACH, *PERIPLANETA AMERICANA* IN SHARKIA GOVERNORATE, EGYPT

By

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### Abstract

In present study, three known species insect parasitic nematodes were recovered from *Periplaneta americana* collected from uptown Zagazig City. These nematodes were 1- *Hammerschmidtella dieingi*, 2- *Leidynema meerutensis*, and 3- *Thelastoma bulhoesi* collected from the hindgut of the host *P. americana*. These parasites were studied by using light microscope, SEM and TEM.

**Key words:** *Periplaneta americana*, *Hammersmidtella* sp., *Leidynema* sp., *Thelastoma* sp., SEM, TEM.

### Introduction

The American cockroach (*Periplaneta americana*), also colloquially known as the water-bug, but not a true water-bug since it is not aquatic (Hall, 2002), or misidentified as the palmetto bug is the largest species of common cockroach (Poertner, 2013). Despite the name, *P. americana* was introduced to what is now know the United States from Africa as early as 1625 (Bell and Adiyodi, 1981). American cockroaches are also known as plagues in the warm The Mediterranean Coast of Spain as well as in southern Spain and southern Portugal (starting from Barcelona to the Algarve) and in the Canary Islands, where the winters are mild/warm and frost-free, and the summers are hot (Copeland, 2003). American cockroaches can become a public health problem due to their association with human waste and disease and their ability to move from sewers into homes and commercial establishments in caves, mines, privies, latrines, cesspools, sewers, sewage treatment plants, and dumps (Komatsu *et al*, 2013). The presence of cockroaches in these habitats is of risky epidemiological significance. At least 22 species of pathogenic human bacteria, viruses, fungi, and protozoans, as well as five helminthic worms' species, were isolated from field collected American cockroaches. Also, they are aesthetically displeasing because they can soil items with their excrement and regurgitation (Baldwin

and Koehler, 2007). In Taiwan, from 250 adult cockroaches, 38 species of gram-negative bacteria, 20 species of glucose non-fermenter bacilli and 6 species of gram-positive bacteria were isolated (Pai, 2003). Besides, in Nigeria many protozoan and helminthes as cysts of *E. hystolitica* and *Balantidium coli*, oocysts of *C. parvum*, *C. cayetenensis* and *Isospora belli*, ova of *Ascaris lumbricoides*, *Anchylostoma deodunale*, *Enterobius vermicularis*, *Trichuris trichura*, and larva of *Strongyloides stercoralis* 234 cockroaches trapped from different human dwellings' sites. In Egypt, cockroaches, apart as others insects, spiders, scorpion, ticks, mites parasitic worms, bacteria, or other living organisms causes Entomophobia or acarophobia, parasitic dermatophobia or delusional parasitosis (Sabry *et al*, 2012). Stankus *et al*. (1990) reported that they have a supplementary role in the spread of diseases, or suspected carriers of the organisms causing: diarrhea, dysentery, cholera, leprosy, typhoid fever, viral diseases such as poliomyelitis as well as eggs of parasitic worms and may cause allergic reactions, including dermatitis,

The study aimed to describe three parasitic nematodes isolated from *Periplaneta americana* collected from indoors and outdoors uptown of Zagazig City, Sharkia Governorate, Egypt.

### Materials and Methods

A total of 160 *Periplaneta americana*

(Linnaeus, 1758) were collected and identified. They were anesthetized with chloroform and then dissected in Petri dishes filled with the normal saline solution under a stereoscopic binocular microscope. Nematode parasites were removed from the hindgut of 119 roaches and picked up with the help of a fine glass dropper. Parasites were washed in normal saline and fixed in hot 70% ethanol. For the study of morphology, parasites were cleared in glycerin and photomicrographed by using Olympus research microscope, and measured with an ocular micrometer.

SEM: the heat killed nematodes were transferred in primary fixed in Karnovsky's fixative for an overnight and then transferred in 2.5% glutaraldehyde. The fixed nematodes were washed with 0.1 M phosphate buffer (pH-7.2) at 4°C and gradually dehydrated in different grades of acetone. The dehydrated specimens were dried by critical point drying method using liquid CO<sub>2</sub> and then mounted on aluminum stubs and gold coated (Green *et al*, 1975). Photography was done using LEO435 VP SEM, Mansoura University and measurements were recorded with LEO-32 annotation program.

TEM: Samples for transmission electron microscopy phoretic or parasitic stages of nematodes were fixed in 1% glutaraldehyde and 0.6% sucrose in 0.1M phosphate buffer (pH 7.4) for more than 24 h. The head or tail regions were cut off and left in the same fixative for more than 24 hrs. After rinsing in the same buffer (six times, 10 min each), the nematodes were post-fixed in 1% osmium tetroxide for 90 min in same buffer. Fixed nematodes were dehydrated in a graded ethanol (50%, 70%, 80%, 90%, & 99.5%). They were cleaned three times with propylene oxide 10min each and infiltrated overnight in a mixture of 50% Eponateresin and 50% propylene oxide and an undiluted resin.

### Results

A total 119/160 *P. americana* were infected with three nematode parasites: 1- *Hammersmiditiella dieingi*, 2- *Leidynema meerutensis*, and 3- *Thelastoma bulhoesi*.

1- *Hammersmiditiella dieingi* female (figs. 1-3): The cephalic extremity rounded. Cuticle striated with rings along entire body. Mouth surrounded by three toothed lips and eight labial papillae. Amphids small and pore shaped. Buccal capsule is short, wide with two mobile teeth on each side of mesostom. Esophagus short and strong, the posterior part of the corpus forms the metacarpus valvate, the anterior and posterior parts of the corpus are differentiated easily, isthmus cylindrical and thin surrounded by nerve ring, ending in a rounded basal bulb heavily muscled and vulvae. Excretory pore is situated at the base of the esophagus. The vulva is slightly protruding and lies in the anterior third of the body. Vagina long and heavily muscled.

Male (figs.4, 5): Body small, truncated in appearance, slightly curved ventrally at the posterior end upon fixation. Cuticle finely striated up to the posterior region of the corpus from the head end. Narrow lateral alae present. Esophagus 0.116mm long, devoid of pseudobulb. Corpus cylindrical, 0.063mm long, 0.007mm wide, isthmus measures 0.036mm in length, 0.007mm in width. End bulb ovate, 0.019mm long, 0.019mm wide, with buccal cavity 2.43x4.8mm. Nerve ring at 0.085mm from anterior end.

2- *Leidynema meerutensis* female (figs.6-9): Body cylindrical, 2.5 mm in length and with a maximum body width of 0.28 mm; head with 2nd annulus much wider and with close-set of 5annuli and then annuli expand abruptly in both, its length and width. First annual with 8 pairs of labial papillae have a surrounding mouth. The cuticle is closely annulated throughout the body length. Lateral alae prominent, each one terminates into a spine-like projection at the posterior. Esophagus is 0.37mm long occupying anterior 14% of body length with a 0.29mm long corpus, 0.020mm long distinct isthmus and an end bulb of 0.11x0.10 mm dimension.

Corpus has two distinct regions, the anterior half is narrow and its posterior part is cylindrical and broader. Intestine prominent-

ly enlarged at the anterior end and is provided with a posteriorly directed much-enlarged characteristic oblong, blind intestinal diverticulum. Vulva transverse in orientation and slightly anterior to middle of body at 1.27 mm from anterior end (V% = 48%).

3- *Thelastoma bulhoesi* female (Figs.10-12): Body relatively robust, widening from base of first cephalic annular, maximum body width at vulva level, directed towards anus. Cuticle annulated from end first cephalic annuli to anus level. Annuli more evident at cervical region level; Annuli of cervical region of cardia (ca) 5µm in width, then increases to ca. 10µm at posterior body half; without lateral alae.

Head with eight paired labiopapillae arranged two sub-dorsal pairs and two sub-ventral pairs. Labiopapillae is C-shaped, elevated, with a deep center. Amphids lateral, as crescent-like pores, located at cuticular elevations top. Buccal aperture triradiate, with three hexagonal labia, arranged as two sub-dorsal and one ventral. Distal side each labia with a rounded cleavage that combines as a circular aperture when 3 labia in contact. First cephalic annule cone-like, truncate, barely inflated, ca. 1.5 head long. Esophagus consists of a muscular, cylindrical procorpus with diameter similar to all its extension. Isthmus is short, slightly slender than procorpus. Basal bulb rounded, valve-plate well developed. Tail subulate, filiform, ending in a fine tip.

SEM of female *T. bulhoesi* (Figs. 13, 14) showed excretory pore, intestine, anus, vulva, anterior annulae and eggs, TEM showed that the cuticle surface has longitudinal incisions. Cuticle layering of body consists of acortical, medial, and basal layer. The external cortex can be resolved into at least five layers (i) a moderately dense outer layer, (ii) a thin electron-lucent layer, (iii) a thin electron dense layer, (iv) a thick moderately dense layer, and (v) a dense inner layer. The internal cortex is relatively homogeneous, although externally it may be of slightly greater density. In addition to nuclei, the hy-

podermis generally includes large numbers of organelles and other structures. The chords are filled with mitochondria (M), sarcoplasmic body (SB) and thin, dense bands (TD) as (Figs.15-18)

### Discussion

In the present study, nematodes were relatively high (74%) in comparison with other nematodes: 5.9% for *Thelastomaalli*, 15.3% for *Leidynema appendiculata* and 20.4% for *Hammerschmidtella indicus* infecting *Periplaneta americana* (Kumar and Singh, 2014). The present study observed a high rate of infection with intestinal parasites in cockroaches (74%). These are similar to the previous studies the prevalence rate of intestinal parasites of cockroaches was less than recorded by Fotedar *et al.* (1991) when they discovered 99.4% of cockroaches collected from hospital and 94.2% cockroaches collected from residential areas were transporting medically important microorganisms and higher than that of Thyssen *et al.* (2004) who reported 58.3% of *Periplaneta americana* carried helminthes.

By several ways helminthes can be carrying to human, but by insects little attention has been given to vector or mechanical transmission of infective forms (Chan *et al.*, 2004). This study reported the importance parasites and role of insects as mechanical vectors of helminthes parasites, the most important role is the arthropods play is in carrying germs and parasites. Cockroaches are arthropod transmitters of disease, playing both as mechanical vectors and as reservoirs of pathogenic agents.

As to *Leidynema appendiculata* Ozawa *et al.* (2014) in Japan reported that the smoky-brown cockroach *Periplaneta fuliginosa* has spread all over the world, and is now one of the most undesired invasive alien pests. Because cockroaches are generally infected by the lastomatid nematodes, they are being distributed around the world with their zoonotic parasitic nematodes. They added that 100% Japanese individuals in contact with *Periplaneta* were infected with this species

*H. diesingi* (Hammerschmidt, 1838) can be distinguished by the nerve ring located anterior to metacarpus, the excretory pore at the base of the esophagus, and in the male by one pair preanal papillae near the anus and a single unpaired ventral papilla near the base of a tail appendage. Sriwati *et al.* (2016) in Japan isolated *H. diesingi* (Hammerschmidt, 1838) was from *P. americana* was recorded in Japan. They added that DIC and SEM confirmed that their specimens agreed with previous records of *H. diesingi* from other countries. Jex *et al.* (2005) reported that *H. hochi* was different mainly by having pear-shaped eggs, the nerve ring is posterior to metacarpus, lateral alae are absent, and tail conoid and subulate. *H. manohari* (Rao, 1958) is characterized by cuticle, striated throughout body length, nerve ring enclosed the metacarpus, and in the male by one pair of preanal, two pairs of adanal and an unpaired median postanal papillae; of two pairs of adanal papillae, one located close to anus and other one well improved, projects laterally from the tail appendage.

### Conclusion

No doubt, cockroaches could be mechanical vector of medical pathogens, and inhabitants of urban centres are at the greatest risk of infection. There must be further analysis of the cockroach and parasite relations.

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### Legend of Figures

Figs.(1-3) Morphology of female *Hammersmiditiella dieingi* showing pharynx (Ph.), pseudobulb (Pb.), Isthmus(Is.), End bulb (Eb.), gastric pouch (G.p.), Intestine (In.), Eggs (E.), ovaries (O.), Anus (A), Tail (T), Buccal capsule (B.C) with different magnifications X, 60 & 120  
 Figs.(4,5) Morphology of male *Hammersmiditiella dieingi* (Chitwood, 1932). Showing pharynx (Ph.), End bulb (Eb.), gastric pouch (G.p.), Intestine (In.), Eggs (E.), Testis(Te), Vesicula seminalis (V.S). X, 80  
 Figs. (6-9) Photomicrographic details of *Leidynema meerutensis* n. sp. (female) (Leidy, 1850). Showing head (H), Oesophagus (O), End bulb (Eb), Intestine (In), Excretory pore (E.P), Intestinal Diverticulum (I.D), vulva (V), Anus(A), Tail (T), Egg(E). X,120  
 Figs.(10-12) *Thelastoma bulhoesi* (de Magalhaes, 1900). Female. Showing Excretory pore(Ep), Posterior bulb (Postb) Nerve Ring (N.R), Pharynx(Ph), Isthmus (Is),End bulb(Eb), gastric pouch (G.p), Intestine (In), Ovaries (O), Uterus (Ut), Anal gland (A.G), Anus (An) with different magnifications X, 60 & 120  
 Figs.(13,14). Scanning electron micrographs of *Thelastoma bulhoesi* (Female). Showing Excretory pore(Ep), Intestine( In), Anus (An), Vulva (V), anterior annulae (Ant.A), Eggs(E).  
 Figs.(15-17): Cross section through lateral field with two incisures showing layering External cortex (Ec), Internal cortex (Ic), medial layer (Md), Basal layer (B), electron- lecent zone (LZ).  
 Fig.(18): Cross section through hypodermal chord showing nucleus (Nu), nucleolus (Nc), mitochondrian (M), sarcoplasmic body (SB) and Thin, dense bands (TD).





