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Occurrence of Intestinal Nematode Parasites in Cockroach, *Periplaneta americana* (L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study investigates the occurrence of intestinal nematode parasites in the American cockroach, *Periplanata americana* (Linnaeus). Thousands of species of insect parasitic nematodes not only attack the general insect form, belonging to different orders but also the insect pests of agricultural, veterinary, medical and forestry importance. Classic identification of nematodes is based on morphological and anatomical differences using microscopic image analysis. Morphological identification is among the cheaper identification methods and helps relate

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morphology with possible function. A total of 200 cockroaches were collected from various locations in Aurangabad city and transported to the Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India for this study. The present communication deals with the taxonomic study of intestinal parasite infection in cockroaches, redescribed of two nematode parasites of *P. americana* were *Hammerschmidtiella diesingi* (Hammerschmidt, 1838) Chitwood, 1932 and *Thelastoma periplaneticola* Leibersperger, 1960 from Aurangabad, (M.S), India. The redescriptions presented in this study enhance our understanding of the morphology and diversity of these parasites, underscoring the necessity for continued research on the intestinal parasite fauna of cockroaches in India.

Keywords: Periplaneta americana; Hammerschmidtiella; Thelastoma; intestinal nematode.

1. INTRODUCTION

"Cockroaches are one of the oldest insect orders with a fossil history extending back more than 300 million years. There are 3500-4000 known species worldwide of which only a few are troublesome to people" (Robertson, 2004). "The American cockroach, *Periplaneta americana* L. is the largest of the common domestic cockroaches measuring on average 4 cm in length. The American cockroach is second after the German cockroach in abundance. The cockroach is found in caves, mines, privies, latrines, cesspools, sewers, sewerage treatment plants, and dumps" (Bell & Adiyodi, 1981; Burmeister, 1838). Cockroaches are recognized to be mechanical vectors of disease-causing agents (Sia et al. 2016). Their presence in these habitats is of epidemiological significance, at least 22 species of pathogenic human bacteria, viruses, fungi and protozoan as well as five species of helminths worms have been isolated from collected American cockroaches (Rust et al*.,* 1991). Children under five years old of age among the group are exceptionally associated with intestinal parasitic infections (Fauziah, 2022).

"Nematodes, a diverse group of roundworms, exhibit a wide range of dietary habits, including parasitism of animals and plants. These parasites cause substantial economic losses in agriculture and pose significant health challenges to humans and animals" (Lin & Siddique, 2024). "Insect parasitic nematodes have been known since the 17th century and perhaps the earliest. Extensive studies on IPNs were carried out in the 19th and 20th centuries. During the last one-decade, remarkable progress has been made in the taxonomy of IPN. Studies on the economic importance and life histories of two mermithid parasites of grasshoppers",
(Nguyen & Smart, 2004).

Agamermis decaudata Cobb et al*.,* 1923 and *Mermis subnigrescens* Cobb, 1926 were excellent contributions and stand as classics in insect nematology (Christie, 1936; Basir, 1956) has done an excellent work on oxyuroid parasites of Arthropods. Poinar (1977) gave a generic key of entomophilic nematodes. Remarkable studies on parasitic nematodes of insects were made by Hammerschmidt (1938, 1947), Leidy (1849, 1853), Cobb (1910, 1930), Travassos (1920, 1958), Steiner (1920, 1930), Artigas (1926, 1929), Chitwood & Chitwood (1937, 1950), Nickle (1963, 1984), and many others.

In India, work on nematode parasites of insects was started by Basir in 1940 and he did a lot during 1940-1970, which was followed by Siddiqi (1960), Farooqui (1967), Duggal and Aulakh (1988, 1989), Singh and Kaur (1988), Singh and Singh (1989), Rizvi and Jairajpuri (1995), Ganguly (2000), Ali (2000) and few others, mainly in North India. In South India, significant contributions and a series of reports were made by Rao (1958, 1965), followed by Kumari (1967), Devi et al*.,* (1991), Reddy and Rao (1987), Hussaini (2003) and others. Very recently Gantait and Chatterjee (2007) reported "30 species of parasitic nematodes of Arthropoda from Andhra Pradesh including two new subgenera and one new species".

"Thousands of species of insect parasitic nematodes not only attack the general insect form, belonging to different orders but also the insect pests of agricultural, veterinary, medical and forestry importance. The potential for the use of entomophilic nematodes as self-perpetuating biological control agents lies in areas where chemical pesticides are too expensive, not practical or most noxious to humans and the environment" (Chitwood et al., 1933). The progress of research in nematode systematics has been particularly impressive in the area of soil nematodes, plant parasitic nematodes and parasitic nematodes of vertebrates. However,

studies on insect parasitic nematodes and their systematics have largely been neglected, though they have enough potential to be used as biological agents to control various insect pests without causing any damage to the ecology (Barron, 1981; Swarup and Gokte, 1986). Thus, with a view to enriching our knowledge on insect parasitic nematodes of Aurangabad, Maharashtra state the present work was proposed.

2. MATERIALS AND METHODS

1. **Collection of the Insects:** Two hundred cockroaches were collected from different parts of Aurangabad city province at night time or in the morning. Each cockroach was collected and put in a sterile test tube and then transported to the laboratory of the Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, (M.S), India.
2. **Isolation Parasites from Internal Surfaces:** After external washing. Cockroaches were placed in flask rinsed with 70% alcohol for 5 min to decontaminate external surfaces then the cockroaches were fixed on dissecting Petri dish and dissected. Collect the nematodes with the fine brush or needles into a small vial of a few of millilitres of ethyl alcohol 70%. Set apart the specimens in a well-stopped vial to examine them later and all separated parasites are stored to be examined later (Walter and Cancun, 2005).
3. **Killing, Fixing, Processing, Entomogenous Nematodes to Glycerin:** There are several standard methods for killing and processing nematodes to glycerin and depending on the type of nematodes good results can be obtained for each. Because of the diversity of insect nematodes, a single simple method cannot be used for all groups.

The method tends to clear the nematode but the definition gradually returns. Nematodes present a special problem during the dehydration process since the body wall is thin and often collapses by fixing TAF made with Ringers indeed of water and using a modification of the glycerin-ethanol method of processing to glycerin good results can often be achieved this method consists of transferring the nematodes from the fixative to a small cavity block contain 5 ml of 20 parts of ethanol (95%), one part glycerin and 79 parts distilled water. This dish is then placed in a closed vessel containing 96% ethanol for 12 hours at 35-40^{0}c. At this time, the dish is filled with a solution consisting of 5 parts glycerin and 95 parts glycerol (96%) and left partially opened Petri dish for 3 hours at 40^{0}c. The nematode then should be in pure glycerin. This method works well for insect nematodes in general, but requires and often the large drenched parasites often collapse (Courtney et al*.,* 1955).

1. **Preparation of permanent slides for taxonomic study:** For permanent mounts, nematodes were first fixed with TAF (2% Triethanolamine and 7.5 % Formaldehyde), dehydrated with a slow evaporation method and mounted on a slide (Morffe and Garcia 2010). Then the nematodes were properly arranged and a cover slip of 20 mm diameter was mounted carefully. Finally, the coverslip was sealed by its edges on the slide by using good quality nail polish as a sealing material. Finally, the slides were properly labeled with the collection data (Chitwood, 1932).
2. **Identification, drawing and microphotographs of specimens:** The identification of Insect parasitic nematodes belonging to various orders up to the species level by observing

The specimens under a compound microscope. The photomicrographs of all specimens were taken by a digital camera attached to the same microscope using low and high-power objectives. (Gantait and Venkataraman 2013).

1. **Use of classification scheme to arrange the available species:** In the present study, classification was proposed by Gantait and Sanyal (2007) and Manjur Shah et al*.,* (2012).

3. RESULTS AND DISCUSSION

3.1 Taxonomy of Insect Parasitic Nematodes in Cockroaches (*Periplaneta americana* L.)

3.1.1 *Hammerschmidtiella diesingi* (Hammerschmidt,1838) Chitwood, 1932 (Fig. 1 and 2)

Host : *Periplaneta americana* Linn.
Habitat : Intestine
Locality : Aurangabad, (M.S), India

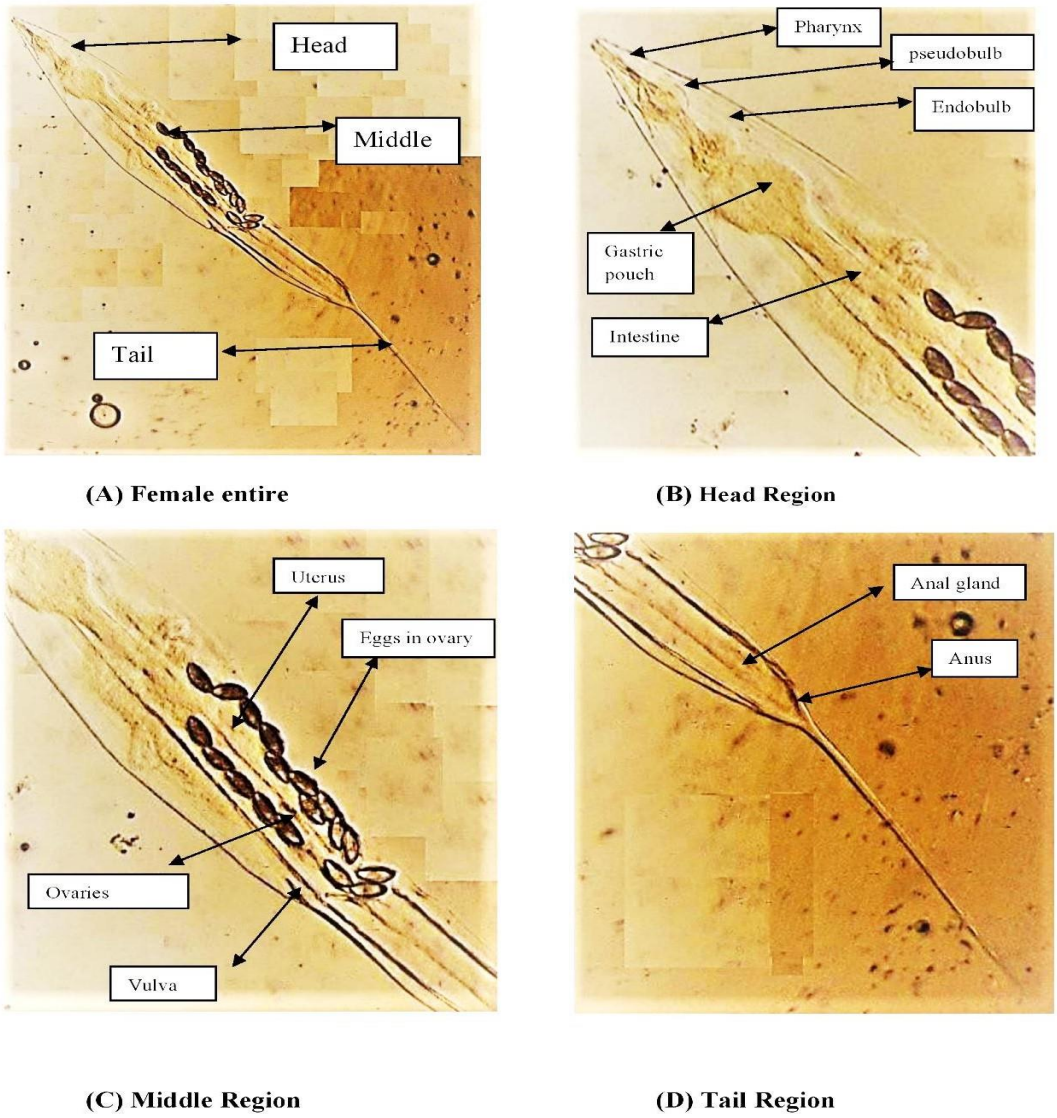

Fig. 1. Hammerschmidtiella diesingi (Hammerschmidt, 1838) Chitwood, 1932

Description: Females **"**(n = 5): L = 2.068 - 3.164 mm; a = 11-12.3 (11.7 ± 0.48); b = 6.4-10.4 (8.7 ± 1.47); c = 2.9-3.2 (2.9 ± 0.13); V = 21.3-25.6 (22.48 ± 1.79) %. Body spindle-shaped. Narrow lateral alae present. Oesophagus consists of a cylindrical procorpus, a large ovoid metacarpus (pseudobulb), distinct isthmus which passes into the valvate bulb. The lumen of the procorpus and the first third of the metacarpus are strong sclerotized. The nerve ring is situated on the posterior end of the procorpus. Excretory pore located at 434-485 µm from the anterior end. Vulva is a transverse slit in the anterior third of the body, posterior to the base of the oesophagus. A long vagina and common uterus posteriorly directed. Gonads didelphic, prodelphic. Eggs elongated, ellipsoidal. One pair of lateral pores at a distance of 20-50 µm after the anal opening. Tail long and filiform" (Nedelchev et al.2013).

Remarks: "Studies of the Bulgarian specimens are consistent with the description and scope of the measurements given by earlier workers who presented their areas of distribution. A cap-like characteristic structure of the tail tip of the female Indian population in our specimens was not observed". (Shah, 2007). Basir (1956a) redescribed "*Hammerschmidtiella diesingi* from Aligarh, Uttar Pradesh". Soota and Chaturvedi (1971) reported "it from the Howrah district of West Bengal; collecting from the rectum and junction of intestine and rectum of *Periplaneta americana*" (thirty Females; Z.S.I.

Reg. Nos. W7069 - 73/1; collector- Y. Chaturvedi, 19- 22. 7. 1967). Gupta & Kaur (1978) reported the species from *P. americana* at Chandigarh, Punjab. Rizvi (2006) redescribed the species from Dehra Dun, Uttarakhand, collected from the posterior gut of *P. americana*. Gantait and Chatterjee (2007a) recorded " it from Anantapur, Andhra Pradesh; collected from t h e rectum of the cockroach host, *Blatta orientalis".*

Distribution: North and South America, North India, China, Russia, Europe (Germany, Nördlingen) (Leibersperger 1960), Poland, North-East India (Shah 2007).

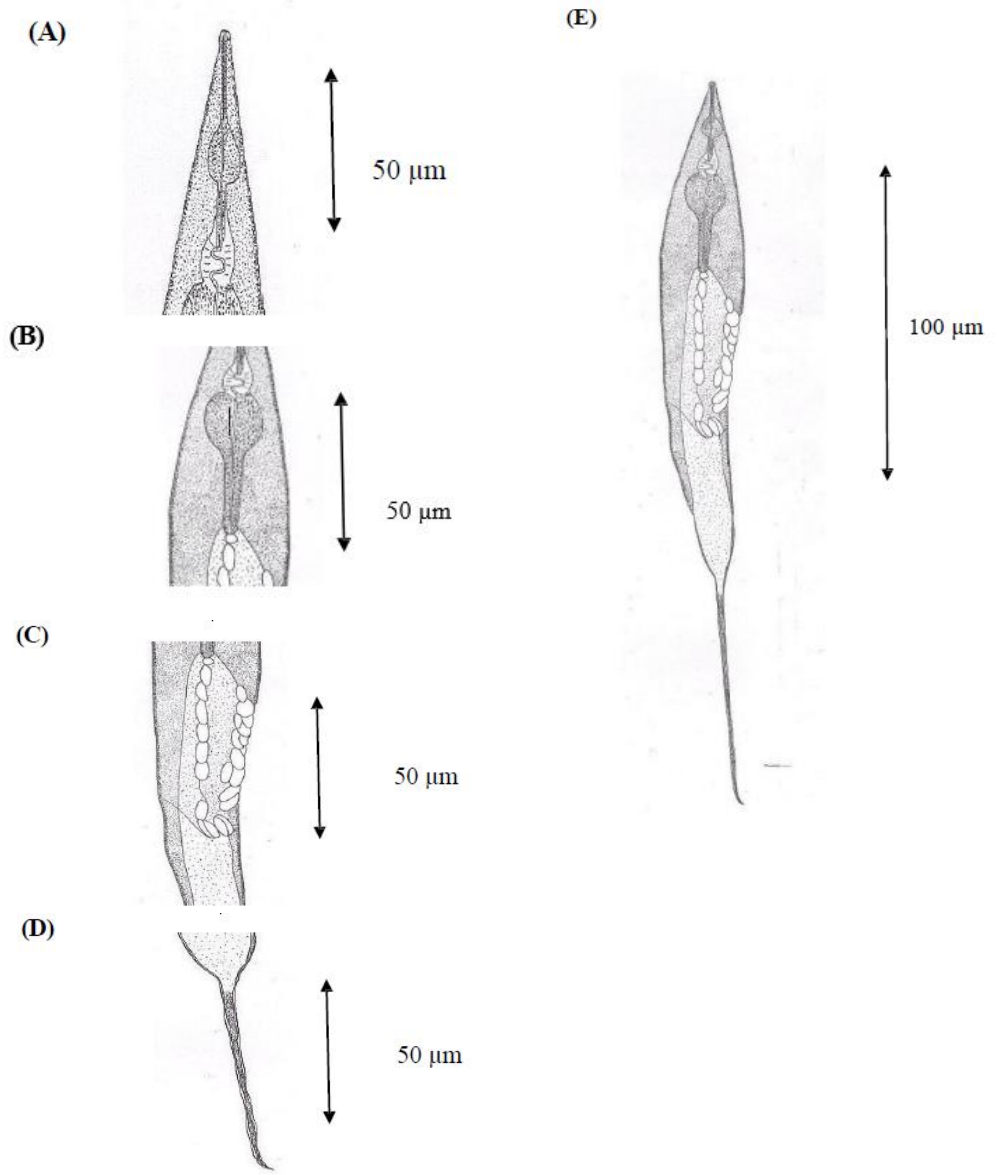

Fig. 2. Camera lucida drawing of *Hammerschmidtiella diesingi* (Hammerschmidt, 1838) Chitwood, 1932

A) Female Anterior end
B) Female oesophageal region
C) Female vulval region showing eggs
D) Female posterior end (lateral view)
E) Female entire

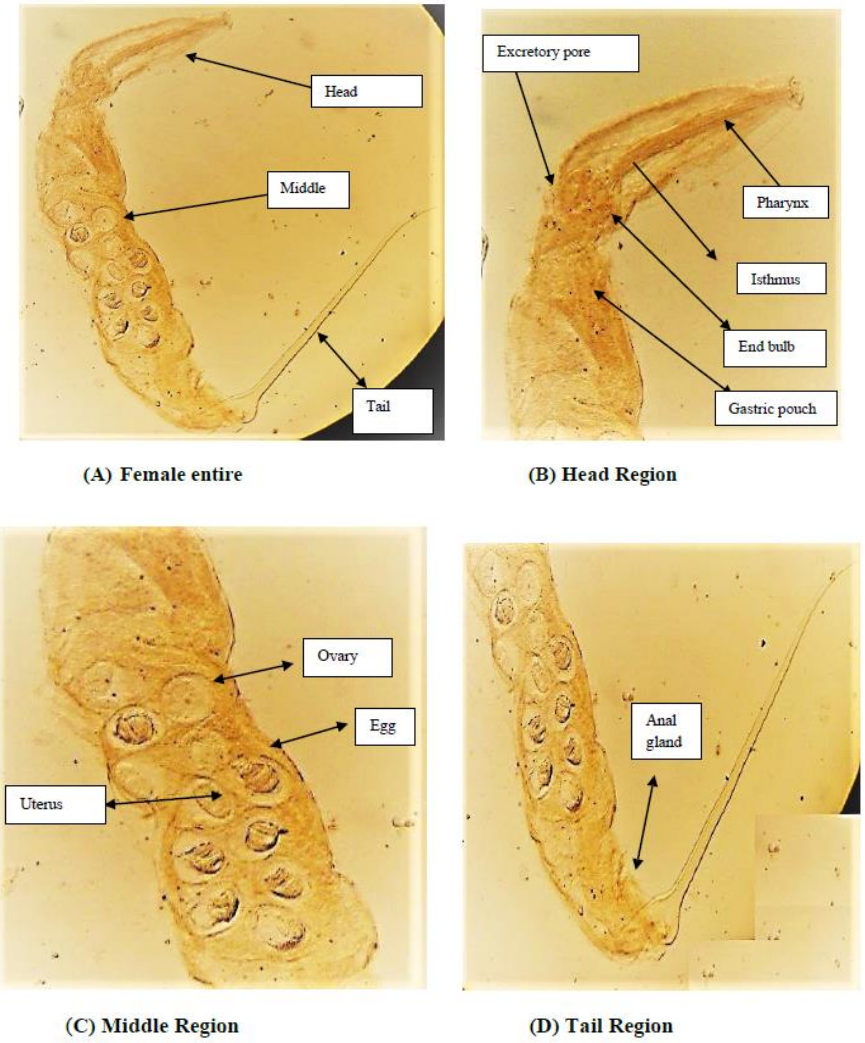

Fig. 3. *Thelastoma periplaneticola* Leibersperger, 1960

3.2 *Thelastoma periplaneticola* Leibersperger, 1960 (Fig. 4)

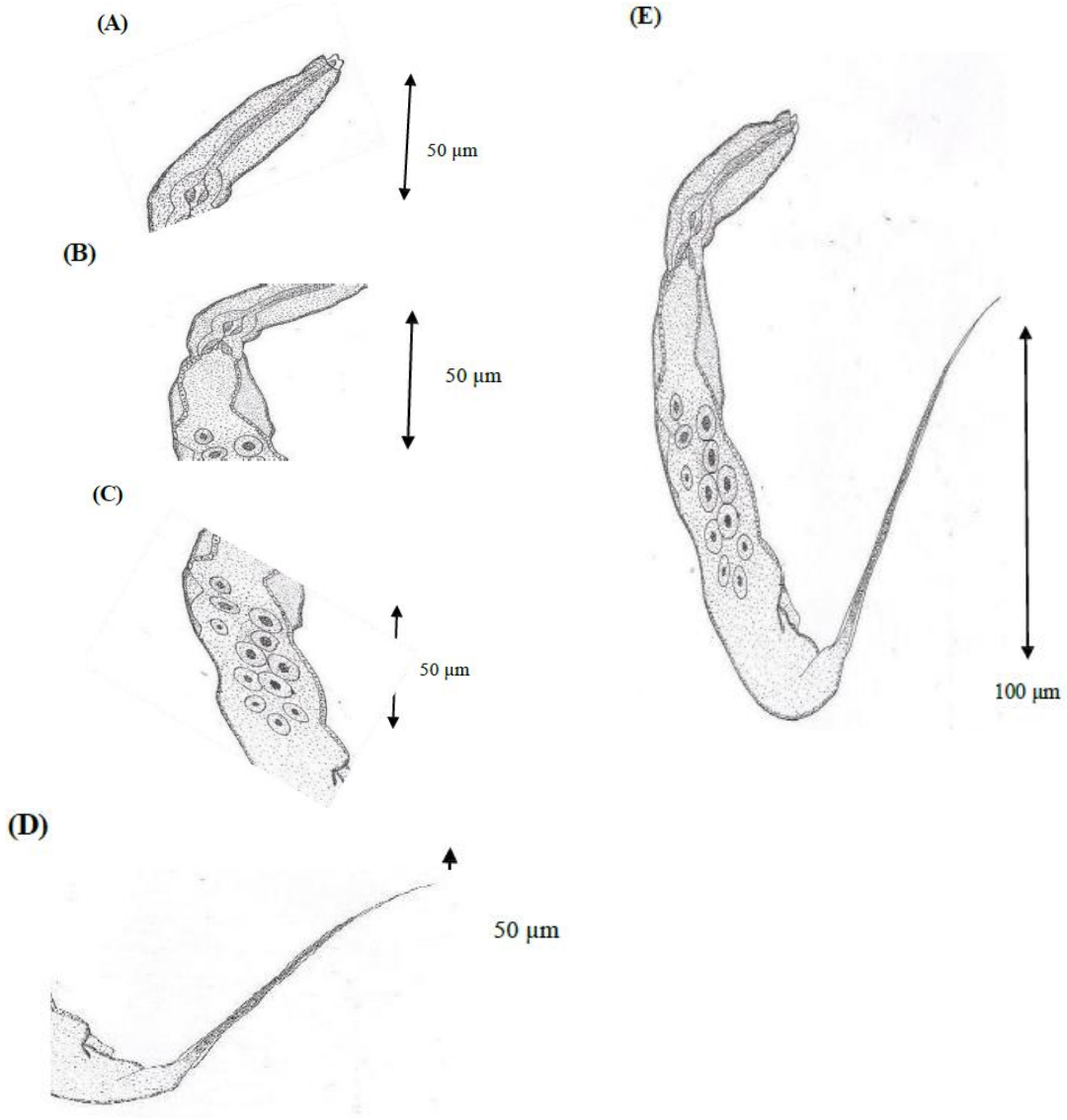

Fig. 4. Camera lucida drawing of *Thelastoma periplaneticola* Leibersperger, 1960
A) Female Anterior end
B) Female oesophageal region
C) Female vulval region showing eggs
D) Female posterior end (lateral view)
Female entire

Host : *Periplaneta americana* Linn.
Habitat : Gut
Locality : Aurangabad, (M.S), India

Genus : *Thelastoma* Leidy, 1849

Description: **"**Females (n = 20): a = 11.05-17.17 (13.206 ± 1.478), b = 5.05-7.20 (5.924 ± 0.686), c = 2.82-3.537 (3.166 ± 0.198), L = 2.138-3.474 (2.655 ± 0.364) mm, W = 0.142-0.284 (0.204 ± 0.0416) mm, oesophagus = 0.402-0.506 (0.447 ± 0.029) mm, excretory pore = 0.343-0.510 (0.417 ± 0.034) mm; nerve ring = 0.170-0.218 (0.194 ± 0.012) mm, buccal cavity = 12.15-14.58 × 12.15 - 14.48 (12.636 ± 0.997 × 13.30 ± 0.619), eggs = 70.47-85.05 × 46.17-60.75 (77.94 ± 4.322 × 55.89± 3.612), tail = 0.657-1.021 (0.838 ± 0.118) mm, vulva = 1.09-1.717 (1.241 ± 0.168) mm. Body small, cylindrical and tapering towards the anterior end

and into a filiform tail posteriorly". (Manjur, 2007). The buccal cavity was distinct. The oesophagus long with a cylindrical corpus, a short isthmus and an end bulb. Excretory pore a little above the base of the oesophagus in females. The vulva a little anterior to mid-body, and vulval lip is well developed. Ovaries amphidelphic, vagina directed anteriorly. The tail is filiform forming one-third of the total body length (Fig. 3D). Eggs are oval in shape (Fig. 3C).

Discussion: The genus *Thelastoma* was erected by Leidy (1849) to accommodate "*T. attenuatum* as its type species. So far, the genus contains 54 (approx.) species described from the world over out of which 19 species are reported from India alone". Thelastoma periplaneticola was first described by Leibersperger (1960) from Germany.

Generic Diagnosis: "Female has Cephalic extremity formed by circumoral annule and enlarged second annule. Mouth surrounded by eight labial papillae. Amphids present. Lateral alae present or absent. Buccal cavity simple. The oesophagus consists of an anterior cylindrical corpus, an isthmus and a posterior valvular bulb. Excretory pore pre- or post-oesophageal bulb or at the level of the base of the bulb. Tail long filiform about one-third to one-fourth of the total body length. The vagina is short, muscular and anteriorly directed with a well-developed vulval lip. Vulva at or posterior to mid-body. Eggs are broadly oval" (Manjur, 2007).

Remarks: All measurements are in conformity with the range given by Leibersperger (1960) except in having somewhat smaller eggs (72-102 × 58-97) and slightly shorter female tails (tail = 0.55-0.94 mm).

According to Shah (2007), the caudal filament tip of female *H. diesingi* from Manipur, India, has a terminal cap-like shape. Later, a number of investigations (Lee 1958; Leibersperger 1960; *Kloss* 1966; Gupta 1997; Blanco et al. 2012) contradicted these findings. This structure (Fig. 1 and 2), where females have a fine tail tip, was not visible to us in our studies. Spindle-shaped body. There are narrow lateral alae. The oesophagus is made up of a wide, oval metacarpus (also known as a pseudobulb), a distinct isthmus that connects to the valvate bulb, and a cylindrical. According to Khairul & Paran (1977), *T. bulhoesi* has a notably shorter tail than *T. bulhoesi* (c = 11.7–23.3 µm), while in the present study, *Thelastoma periplaneticola* has a longer tail which is closed to *T. dollfusi. T. malaysiense.*

4. CONCLUSION

In conclusion, the present taxonomic study confirms the presence of two nematode parasites, *Hammerschmidtiella diesingi* and *Thelastoma periplaneticola*, infecting the intestines of *Periplaneta americana* from Aurangabad, Maharashtra, India. The redescriptions provided in this study contribute to a better understanding of the morphology and diversity of these parasites, highlighting the need for further research on the intestinal parasite fauna of cockroaches in India.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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