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Original paper

# Scanning electron microscopy of *Cysticercus fasciolaris* (larval *Taenia taeniaeformis* Batsch, 1786) from the wild rat, *Rattus rattus* Linnaeus, 1758, morpho-physiology and risk to human health

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**ABSTRACT.** Autopsy of *Rattus rattus* (n=65) revealed 1–7 creamish to white cysts, 2–7 mm in diameter embedded in the liver parenchyma of 12 rats. Dissection yielded 45–65 mm×5 mm sized segmented strobilocercii of *Cysticercus fasciolaris* (larval *Taenia taeniaeformis*) coiled inside. Light microscopy indicated large scolices, the rostellum armed with four suckers and one row of taenoid type hooks having long blunt handle with sharp pointed blade, other row of hooks was in the developing stage. The strobila lacked genital organs and ended in a tail-bulb suggesting it was juvenile. SEM showed anterior crown of 19 hooks and poorly developed posterior crown. A collar (66.0–86.0 µm in width) armed with papillae (30.2–35.4 µm base and 12.5–14.5 µm tip) and pores (17.0–22.4 µm in diameter) surrounded the hooks. The body segments were 125–145 µm in diameter, at the tail bud, 380–410 µm in diameter. The segments were armed with pores, 11.5–14.5 µm in diameter. Netted (2.5–4.5 µm wide and of varying lengths) body segments provide flexibility to the worm during attachment. The tail bud was 2.4–2.7 mm in length and 1.5–1.71 mm in width. The SEM data presents a significant advancement over light microscopy and the morphological features generated herein can safely be utilized to correlate with the parasite's physiological functions. This is the first report of *R. rattus* as a natural intermediate host of *T. taeniaeformis,* and may pose serious risk to human health in urban areas of Bareilly, India and merits attention.

**Keywords:** scanning electron microscopy, *Cysticercus fasciolaris*, *Rattus rattus*, hooks, *T. taeniaeformis*

## Introduction

*Taenia taeniaeformis* (Batsch, 1786) Wolffügel, 1911 is a parasite characterized by cosmopolitan geographic distribution. The final hosts are carnivores of the families Felidae, Canidae and Mustelidae, including domestic cats and dogs [1] and the intermediate hosts are mouse, rat, muskrat, squirrel, rabbit, other rodents, bat and humans. Cats acquire *T. taeniaeformis* infection from scavenging rodents in which the larval stage, i.e. *Cysticercus fasciolaris* is encountered [2]. Rodents generally acquire infection with contaminated water, food or bedding infected with cat faeces. In the intermediate host, *C. fasciolaris* cysts are found as multiple hepatic cysts. Gupta et al. [3] observed haemato-clinical changes in pregnant and non-pregnant *Rattus rattus* infected with parasites and Gupta et al. [4] recorded changes in condition factor and organosomatic indices in parasite infected *Rattus rattus*.

Scanning Electron Microscope (SEM) is a powerful magnification tool that utilizes focused beams of electrons providing topographical, morphological and compositional information. Al-Jashamy and Islam [5] studied the morphological features of *Taenia taeniaeformis* under SEM, clearly differentiating it from other Taeniae species. The

metacestodes of *C. fasciolaris* were found in the liver of the wild rats, *Bandicota bengalensis* [6]. Karim [7] employed scanning electron microscopy (SEM) to observe the morphology of the parasite in male Sprague-Dawley laboratory rats from Malaysia. During the present course of investigations, scanning electron microscopy was utilized to observe the surface topography of *C. fasciolaris* isolated from the Indian wild rat, *Rattus rattus* in order to provide insight to morphological details and correlate them with their adaptive potentialities.

Clinical signs due to *Taenia taeniaeformis* infection vary from unthriftiness, malaise, irritability, capricious appetite, and shaggy coat to colic and mild diarrhea; rarely, intussusceptions of the intestine, emaciation, and seizures are seen.

The rats infected with *C. fasciolaris* were weak and dull and had rough hair coat. In general, the intermediate host shows many more signs of illness than the definitive host [9]. Affected rats were underweighted, lethargic, emaciated, and reluctant to move and touch, showed weight loss, anorexia, distended abdomen with presence of tapeworm larvae in rat liver are suggestive of pathogenesis and ill health of rats which are commonly used as laboratory model for various biomedical researchers. Their mucosal membranes were pale and among abdominal organs, only their spleens were much smaller than their healthy counterparts. Metastatic hepatic sarcoma has been reported in rats due to infection of cysticercus of *T. taeniaeformis* [7,10,11]. Non specific signs like decrease in serum cholesterol and glucose, with increase in serum alanine aminotransferase, sorbitol dehydrogenase, neutrophils etc. are observed where lymphocytes and eosinophils were also found associated with infection [10].

The larval stage was first described as *Cysticercus fasciolaris* Rudolphi, 1808. The genus, *Hydatigera*, was established for this larval form. Küchenmeister [12] was the first to show that the larval stage in rodents could infect cats, and Leuckart [13] completed the entire life cycle, with infection of rodents with eggs and infection of cats with the larval stage in rate, in the laboratory. In the attempt to divide the genus *Taenia* on the basis of various life cycle patterns, the species in the cat, *taeniaeformis*, was assigned to the genus *Hydatigera*, while other species were assigned to *Taenia*, *Multiceps*, *Taeniarhynchus*, etc. Although this division of the genus has some merit, it was questioned by Verster [14] who felt that this division is unwarranted.

Previously, the parasite was referred to in the adult stage as *Taenia crassicollis* and in the larval stage as *C. fasciolaris*, *H. fasciolaris*, *S. fasciolaris* and *C. taeniaeformis* [7,15,16]. The larval form is mainly localized in the rodent liver and experimental studies have indicated the relationship between implantation of the larvae and development of neoplasms in the liver [17]. However, tumor formation was not observed in any of the specimen.

Shukla et al. [18] discussed behavioral, histological and biochemical effects of *C. fasciolaris* in albino rats. Singh and Arya [19] explained mortality in wistar rats due to the infection *C. fasciolaris* and *Hymenolepis diminuta*.

The present study attempts to place on record the ultrastructure of *Cysticercus fasciolaris* through SEM and its relevance with respect to human health.

## Materials and Methods

Wild rats (n=65) (weight 140–280 g) were trapped alive from storehouses and godowns of different areas of Bareilly, U.P. India, immediately transported to the laboratory, maintained in rat cages and fed on food grains, bread crumbs and water *ad libitum*. To avoid the effect of stress on the body and other parameters, rats were properly handled, anaesthetized and dissected with minimal pain to the animal according to ethical guidelines. They were examined preferably within 24 hours from capture.

The visceral of abdominal cavity including the gastrointestinal tract, kidney and liver were grossly checked for the presence of *C. fasciolaris* cysts. Examination of the liver revealed the presence of encysted cestodes (Fig. 1A). Multiple hepatic cysts of different sizes were observed on the liver, they were carefully removed, teased and the larval cestode extracted and relaxed in saline water.

The cestodes were washed with distilled water several times and fixed in 10% formalin or Carnoy's fluid (absolute alcohol 60 ml; chloroform 30 ml; glacial acetic acid 10 ml). Care was taken to kill and fix cestodes properly so that they remained elongated and properly dorsoventrally flattened between two slides, tightly thread-wrapped and pressed gently so that the internal organs of the worms were not distorted. Correctly fixed and flattened worms were then stored in 70% alcohol in labeled glass vials. The parasites were grossly examined, squash preparations of strobilocerca were

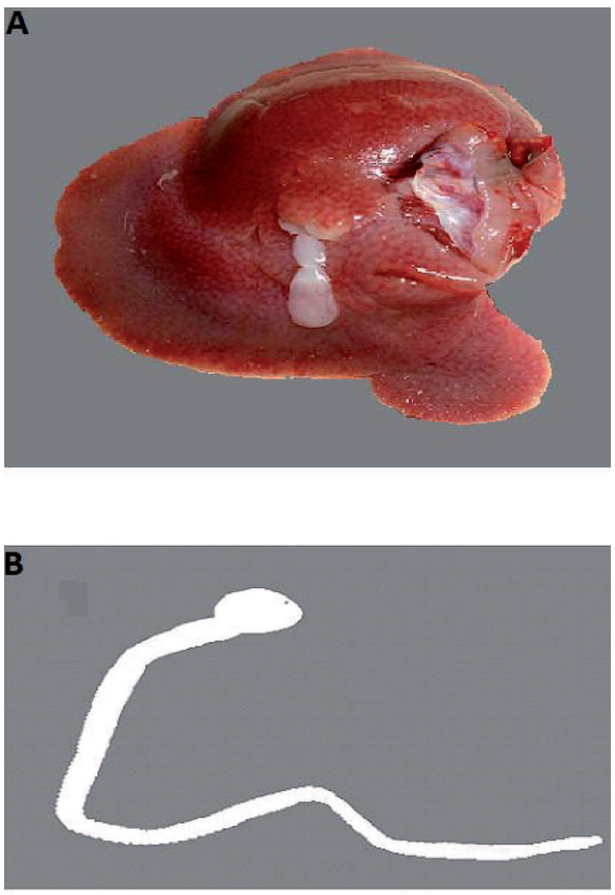


Figure 1. *Cysticercus fasciolaris* from *Rattus rattus* embedded in liver (A) and extracted and isolated (B)

made by clearing in lactophenol and examined under a light microscope. Photographs were taken under Olympus BX-53 microscope and the parasites measured with Cellsens software imaging system.

Scanning electron microscopy of *Cysticercus fasciolaris* was performed in the "Centre of Excellence" Laboratory of Department of Animal Science, MJP Rohilkhand University, Bareilly according to Gupta [8]. Parasites were collected from the host as given above, washed with 0.1M Phosphate Buffer solution, fixed in 2% glutaraldehyde solution buffered with 0.1M phosphate buffer for 15–20 hours, washed in 0.1 M phosphate buffer solution for 30 minutes, so that the smell of glutaraldehyde disappeared. The parasites were dehydrated through graded ethanol series from 50% to 100% ethanol for 10 minutes in each, air-dried, gold-coated for 30 seconds in a Smart Gold coater, placed in the SEM and examined in JEOL Neoscope, the image being projected on a computer screen.

## Results and Discussion

The liver of 12 out of 65 autopsied rats (weight 140–280 g) revealed creamish to white cysts (2–7 mm) in diameter embedded in their parenchyma. The cysts enclosed in a well-defined capsule wall liberated the metacestode on slight teasing (Fig. 1A). The number of cysts in a single liver varied from 1 to 7. It might be possible that infection gradually increased due to continuous exposure to *T. taeniaeformis* eggs. When the cysts were dissected, white to creamish coloured segmented *strobilocercii* were found coiled inside, which were carefully teased taking out the entire parasite in normal saline (Fig. 1B). The morphology of the strobilocercii and their anatomical site of predilection in rats confirmed them to be morphologically consistent with larva of *T. taeniaeformis* as per structure and pattern of taenoid hooks and were thus identified as *C. fasciolaris* (larval *T. taeniaeformis*).

The parasites were 45–65 mm×5 mm in size having no fluid. The wall was thin but fibrotic. Individual strobilocerca were opaque white and lodged in close curvilinear arrangements. Their identity was revealed upon opening the cysts, when they burst out. Squash preparations of strobilocerca, cleared in lactophenol for examination under a light microscope, revealed that they were still immature. The scolices were large, the rostellum was armed with four suckers and typically one row of taenoid type hooks having long blunt handle with sharp pointed blade, the other row of hooks was in the developing stage. As the worm was juvenile, the segmented strobila lacked genital organs. The posterior portion ends in a tail-bulb.

The attachment elements (hooks and suckers) have since long been used as valid taxonomic tools for differentiating subgenera and species of cestodes. It is commonly used to distinguish species that appear morphologically identical when examined under light microscope.

SEM studies revealed that the parasite had a well-developed anterior crown of 19 hooks but the posterior crown was still in the formative stage (Fig. 2A) suggesting that the parasites collected were young parasites. The suckers were anterio-lateral in position (Fig. 2B). The size of the anterior crown of hooks was 160–185 µm×25.0–32.0 µm (Fig. 2C–F), posterior 95–110 µm×16.0–22.0 µm indicating their formative stage. The rows of hooks were surrounded by a collar (66.0–86.0 µm in width) which were armed with papillae (30.2–35.4 µm base

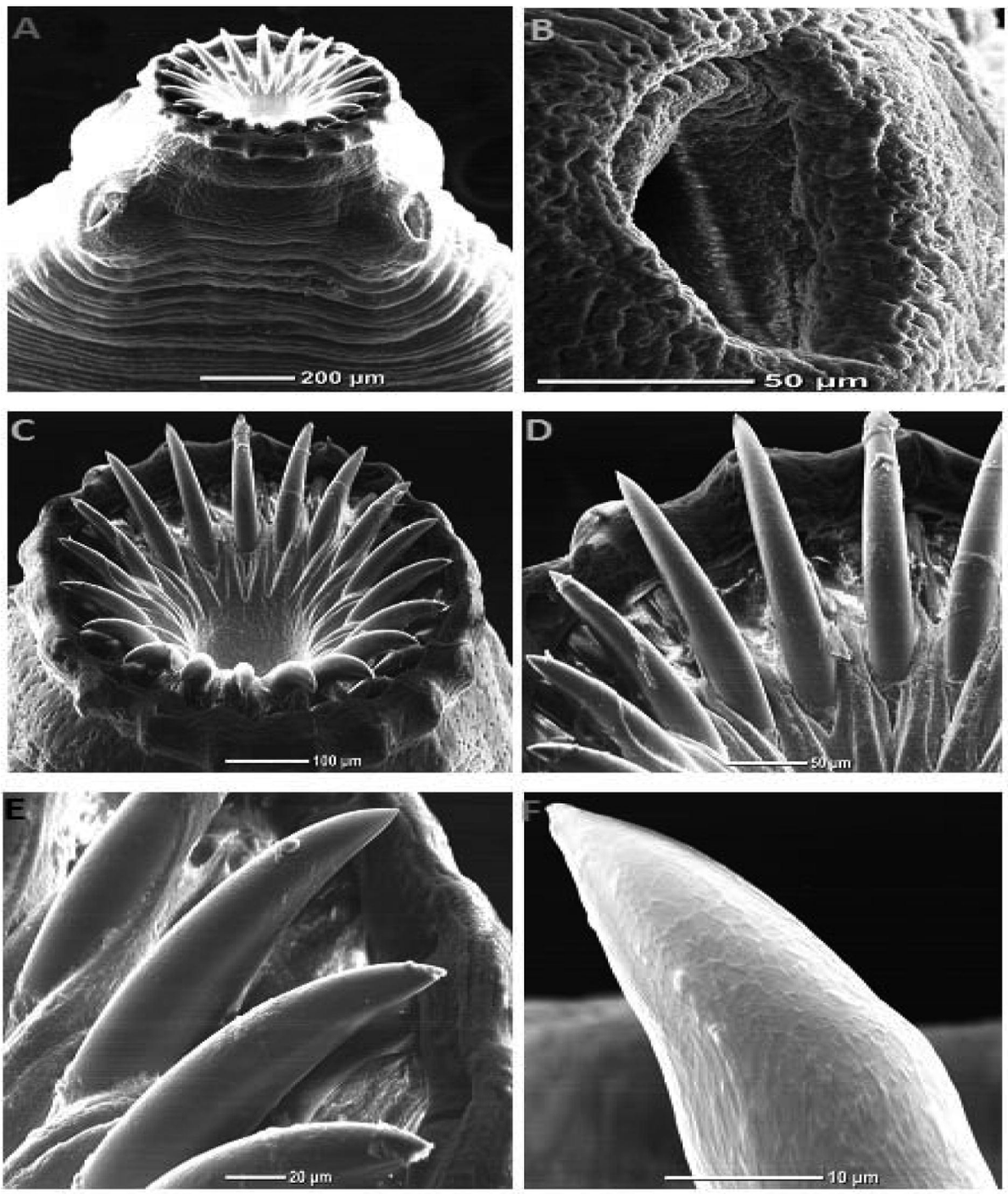


Figure 2. Scanning electron microscopy of anterior end of *Cysticercus fasciolaris*. (A) anterior end showing scolex and suckers, (B) single sucker magnified, (C,D) scolex showing anterior end of fully formed hooks and posterior end of developing hooks, (E,F) anterior hooks magnified

and 12.5–14.5 µm tip) and pores (17.0–22.4 µm in diameter). The body segments were 125–145 µm in diameter but as they approached the tail bud, they became wider reaching 380–410 µm in diameter (Fig. 3A,B). The segments were armed with pores, 11.5–14.5 µm in diameter (Fig. 3C,D). The body segments had net-like structures (2.5–4.5 µm wide and of varying lengths) which provide flexibility to the worm during attachment (Fig. 3E). The tail bud was 2.4–2.7 mm in length and 1.5–1.71 mm in width (Fig. 3F).

Morphometrics of hooks and suckers are of key significance in the discrimination of closely related species of cestodes. Congeneric species are often

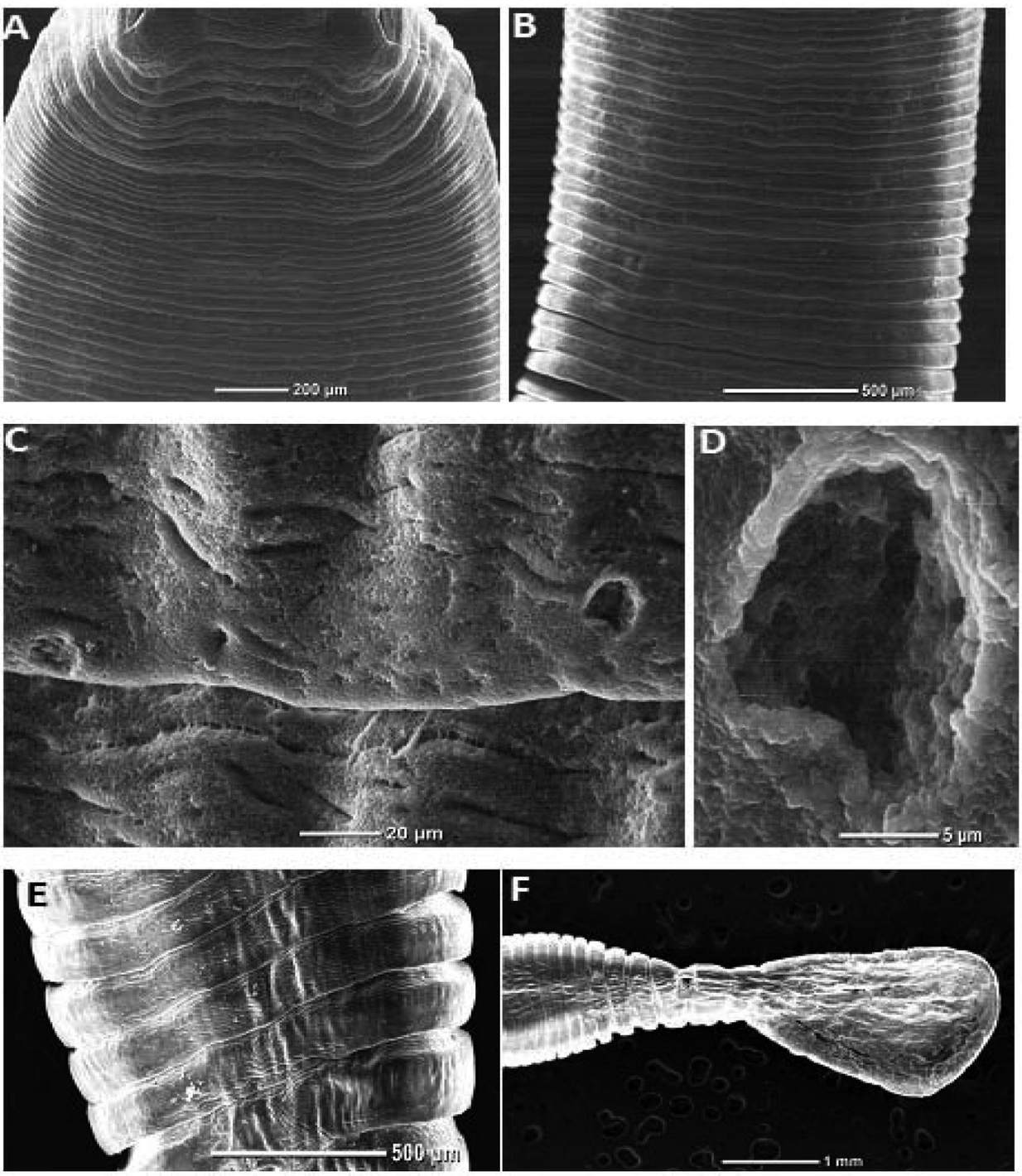


Figure 3. Scanning electron microscopy of proglottids and tail bud of *Cysticercus fasciolaris.* (A) anterior body segments, (B) mid body segments, (C) body segments at higher magnification with pores and net-like processes, (D) pore magnified, (E) wider posterior body segments, (F) posterior end

differentiated only on the basis of subtle differences in hook armature. The hooks vary in size and shape, show morphometric variation from apex to base and can be measured easily, therefore studies on hooks and suckers of cestodes are of prime importance as their number, arrangement and structure are important interspecific diagnostic tools used in taxonomy and classification. Hooks assist in securing the worm into the gut wall and the suckers play a role in attachment. In later stages, the hooks may damage the tissue epithelium. However, detailed information on their ultra-structure is still wanting. In a mature worm, the rostellum is armed with double and alternating rings of large and small

hooks, arranged in a circular pattern with a large double circlet of 30–40 hooks [6]. Malsawmtluangi et al. [20] reported double row of hooks in the metacestode, *Cysticercus fasciolaris* from *Rattus rattus* too. However, SEM studies in the present case showed that although the metacestode had a well-developed anterior crown of 19 hooks but the posterior crown was still in the formative stage (Fig. 2C,D) indicating its juvenile nature.

The outer body wall of tapeworms consists of a tegument. It is the host-parasite interface, and metabolically active body covering performing all the vital activities such as protection, absorption and secretion. The tapeworms have no digestive system. Therefore, the tegument plays an important role in nutrient absorption. The tegument even absorbs some of the host's own enzymes. These enzymes help in digestion. The glycocalyxis is responsible for inhibition of the host digestive enzymes, absorption of cations and bile salts, and enhancement of the host amylase activity [21]. The acidic glycosaminoglycans of the glycocalyx are specific for inhibiting a number of digestive enzymes of the host [22]. The microtriches in cestodes, and pits and spines in trematodes increase the surface area of the teguments for enhanced absorption of nutrients. In addition, they act as sensory organs for detecting the surrounding environmental cues. The capacity of the tegument to absorb exogenous materials is proportional to the number and extent of pits or microtriches and the number of mitochondria in the distal cytoplasm [23].

Since cestodes are devoid of digestive and excretory systems, the tegument constitutes the principal site of absorption of nutrients and elimination of waste materials. In fact, the tegument highly resembles the gut of animals turned inside out [24]. A large number of important enzymes have also been detected in the tegument. Glutathione S-transferase, ATP diphosphorylase, lkaline and acid phosphatases, β-glucorunidase, aminopeptidase, acetylcholine esterase, phosphofructokinase, glucose transporters, serine hydrolases and several glycolytic enzymes with their biological roles [25] which probably help in the digestion of food probably to compensate for the lacking alimentary canal.

Abella et al. [26] recorded proliferation of submucosal glands and severe lymphoid follicular hyperplasia in duodenum of rats infected with larvae of *T. taeniaeformis.* Immunofluorescence assay of fibrosarcoma in liver sections showed concurrence with spindle cell sarcoma [27]. The suckers present on the scolex are able to absorb materials not available to other body segments, but absorption mainly occurs throughout the body segments, characteristic of cestode physiology [1].

Al-Jashamy and Islam [5] reported the presence of micropapillae on the top surface of rostellum of adult *T. taeniaeformis* collected from the cat. However, the present worm was devoid of this ornamentation. This is the first record and study on *Cysticercus fasciolaris* from *Rattus rattus* of Rohilkhand, Uttar Pradesh, India using electron optics. Based on the present results, we propose that the newly recognized morphological features as revealed by SEM data presenting a significant advance over light microscopy are recommended as taxonomic criteria and can safely be utilized to correlate with the parasite's physiological functions.

This work is the first report of *R. rattus* as a natural intermediate host of *T. taeniaeformis* in urban areas of Bareilly, India. The strobilocercus larvae were recovered from hepatobiliary system of *R. rattus*. This species has been incriminated as zoonotic with medical importance [28]. It was observed during the study that there has been an increase of human activities in Bareilly, India. Dogs and cats were also seen in this area, it's likely that these animals may pick up zoonotic parasites from wild surface dwelling or subterranean rodents and introduce it to humans and laboratory animals in the nearby regions. The studies pave way for further investigations to evaluate the risk of zoonotic disease transmission to humans in this locality in view of the increased human encroachment into this region.

In conclusion, the SEM data on *Cysticercus fasciolaris* presents a significant advancement over light microscopy and the detailed morphological features generated herein, specially with reference to the hooks (attachment organs) and body surface can assist in correlating with the parasite's physiology for attachment to host tissues and absorption of nutrients. Observations on clinical features of parasite manifestation and risk to human health are suggestive of zoonotic implications. This is the first report of *R. rattus* as a natural intermediate host of *T. taeniaeformis* in urban areas of Bareilly, India and merits attention.

In conclusion, further studies involving other populations and species of Felidae, Canidae and Mustelidae are recommended to determine if these species serve as reservoirs of *T. taeniaeformis* within its distributional range throughout India.

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