

# HISTOLOGICAL STUDIES ON THE ALIMENTARY CANAL OF THE MILLIPEDE, *ANOPLODESMUS TANJORICUS* (POCOCK), (DIPLOPODA: POLYDESMIDA)

S. V. DESHMUKH\* AND C. K. DESHMUKH<sup>1</sup>

Department of Zoology, R. D. I. K. and K. D. College, Badnera - 444 701, Amravati

<sup>1</sup>P. G. Department of Zoology, Amravati University, Amravati - 444 606

E-mail: sharadavd@gmail.com

## KEY WORDS

Peritrophic membrane  
Pylorus  
Intima  
Oesophageal valve  
Sheath layer

Received on :  
03.08.2011

Accepted on :  
07.11.2011

\*Corresponding author

## ABSTRACT

Histologically the wall of alimentary canal of *Anoplodesmus tanjoricus* is composed of five cellular layers, progressing internally these are, sheath layer, longitudinal muscle layer, circular muscle layer, basement membrane and columnar epithelial layer. Foregut and hindgut are lined internally with cuticular intima, however, mid gut is lined internally with peritrophic membrane. Sheath layer is absent in the foregut region. Oesophageal valve is present between the foregut and mid gut which possibly regulates the passage of food from the foregut into the midgut. Sheath layer is highly developed in the mid gut epithelium. Bundles of muscle fibres are embedded in the sheath layer. These bundles form longitudinal thickenings on the surface of the mid gut wall. Circular muscles are poorly developed. The columnar epithelial cells presents distinctive brush border of microvilli. In the posterior region, the mid gut epithelium shows folding and cuticular intima is also seen. Circular muscles are well developed, sheath layer is reduced and longitudinal muscle bundles are seen outside the circular muscle layer. Pylorus marks the junction of mid gut and hindgut. Hindgut is divisible into ileum, colon and rectum. In this region, circular muscles are well developed, longitudinal muscle bundles are distributed unevenly around the circular muscle layer and sheath layer is absent.

## INTRODUCTION

Millipedes are found in damp and humid conditions under rotten wood, old trees and soil litter. They feed on decaying vegetable matter soil and soil litter. They are the essential animals of the forest floor. Histology of alimentary canal of millipedes have been successfully studied by Hefner (1929); Miley (1930); Nunez and Crawford (1977); Krishnan (1968); Shukla (1981); Kumar (1983).

In India millipedes received less attention as compared to other arthropod groups. Despite their large availability they are least explored in India. The genus *Anoplodesmus* is represented by only 4 species in India (Attem, 1936). But surprisingly there appears no histological work on this species. Thus, present investigation was undertaken to study histology of alimentary canal of the millipede *Anoplodesmus tanjoricus*.

*Anoplodesmus tanjoricus* is a polydesmid millipede found abundantly in Amravati District (Maharashtra, India) in the months of July to December. It is vegetarian and inhabits cool, moist localities, cow -shades, dung-pads, fields and under rotten wood where there is plenty of organic matter to devour. During rainy season, they show swarming behavior and enter into the houses of the villagers that prove nuisance to them.

## MATERIALS AND METHODS

Prior to histological study of alimentary canal, the animals

were separated from culture medium and starved for 24 h. They were anaesthetized with cotton pads soaked in chloroform, fixed in small wax trays and dissected in cold Ringer's solution under stereoscopic binocular microscope. The alimentary canals were removed carefully and rinsed in cold Ringer's solution to remove the adhering tissue and gut contents. The alimentary canal was cut into foregut, anterior mid gut posterior mid gut, pylorus, ileum, colon and rectum. The tissues were fixed in aqueous Bouin's fixative for 12 to 24 h. After fixation, the tissues were washed in running tap water overnight to remove the fixative completely. Then the tissues were dehydrated through graded series of alcohol, cleared in xylene, embedded in paraffin wax (melting point 58°C to 60°C). The sections were cut at 4-5 $\mu$  thickness and mounted on albuminized slides and were stained by double staining method using iron haematoxylin and eosin.

## RESULTS AND DISCUSSION

Histological observations on the wall of alimentary canal show that, it is composed of five cellular layers. Progressing internally these are – the outermost layer is the sheath layer which is made up of irregularly spaced cells with large nuclei; a longitudinal muscle layer; a well developed circular muscle layer consisting of several sheets; a basement membrane, penetrated with tracheoles and muscle fibers; the innermost columnar epithelial layer.

The inner surfaces of foregut and hindgut including pylorus

and rectum are lined with cuticular intima. However, mid gut is lined internally with peritrophic membrane.

Although, a number of specimens from different collection sites were examined, their gut proportions and anatomy appears to be mostly similar. Therefore, the following observations of anatomical aspects of the gut regions were based on a single specimen measuring about 42 mm in length and with a diameter of 5 mm.

**Foregut :** The foregut (Fig. 1) is slender, yellowish tube about 5 mm in length and 1 mm wide. The width of the foregut remains relatively uniform throughout the length, except for the posterior end where it slightly enlarges. The foregut epithelium is lined on its lumen facing surface by a chitinous intima. The epithelium consists of columnar cells measuring about  $18.5 \mu$  to  $148 \mu$  in length. The epithelial cells show brush border. The nuclei are avoid and are present at the bases of these cells. A basement membrane surrounds the basal surface of the epithelium. The foregut epithelium forms six definite folds in the lumen of the foregut that occupy most of the luminal cavity. In the middle portion of the foregut, these six triangular folds give the lumen of the foregut a star shaped appearance. At the posterior end of the foregut, these folds ramify and give slender branches. The basement membrane underlying the

epithelium follows these folds but the muscles do not enter these folds. The longitudinal muscle fibers are interspersed in the matrix of the basement membrane.

The foregut musculature consists of  $7.4 \mu$  to  $14.8 \mu$  thick circular muscle layer which envelops the epithelium completely. Numerous longitudinal muscle fibers are seen outside the circular muscles. Posteriorly the foregut projects into the anterior lumen of the mid gut forming an oesophageal valve. This valve possibly regulates the passage of food from the foregut into the hindgut (Fig. 2).

**Midgut:** It is characterized by the absence of cuticular intima lining the epithelium. A thin peritrophic membrane is found in the lumen of the mid gut.

The mid gut musculature is well developed in the millipede, *Anoplodesmus tanjoricus* (Fig. 3). The sheath layer surrounding the mid gut epithelium is highly developed and consists of large cells forming a network. Presence of bundles of muscle fibers embedded in the sheath layer is the characteristic feature of mid gut wall. These bundles form longitudinal thickenings on the surface of the mid gut wall.

Circular muscles are poorly developed but can be seen as a continuous layer. The basement membrane is very thin and

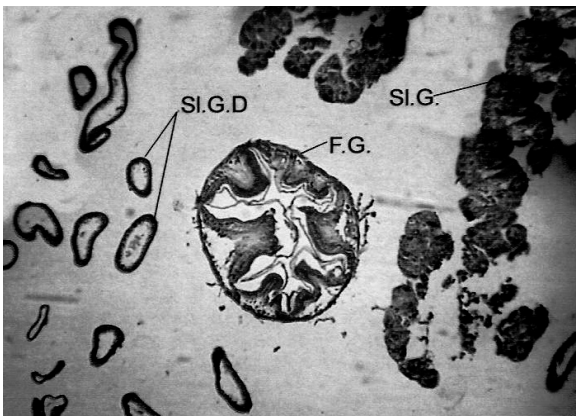


Figure 1: T. S. of anterior region of foregut showing, salivary glands and salivary ducts (H.E. x 100)

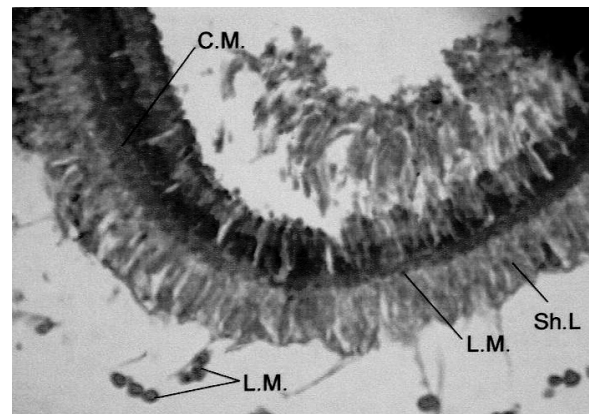


Figure 3: T. S. of alimentary canal passing through middle region of midgut showing columnar nature of epithelial cells. Fragmentation of cells is also seen (H.E. x 100)

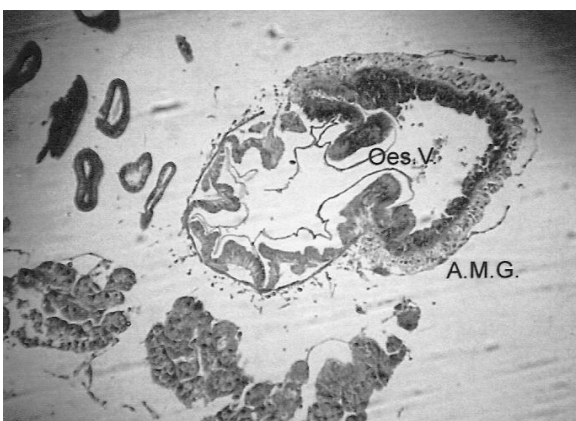


Figure 2: T. S. of alimentary canal at the region of foregut and midgut, showing oesophageal valve projecting into the midgut (H.E. x 100)

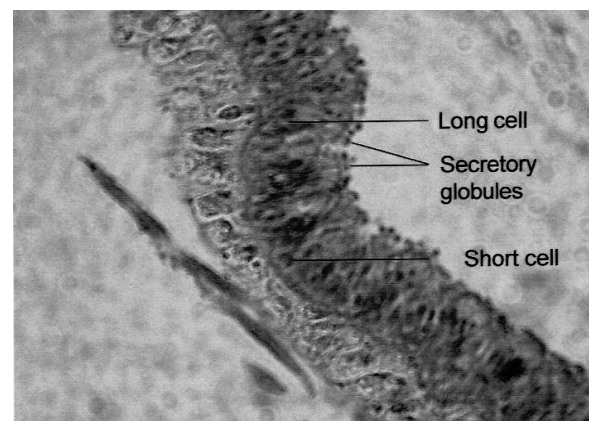


Figure 4: T. S. of alimentary canal passing through middle region of midgut showing long cells, short cells and secretory globules (H.E. x 100)

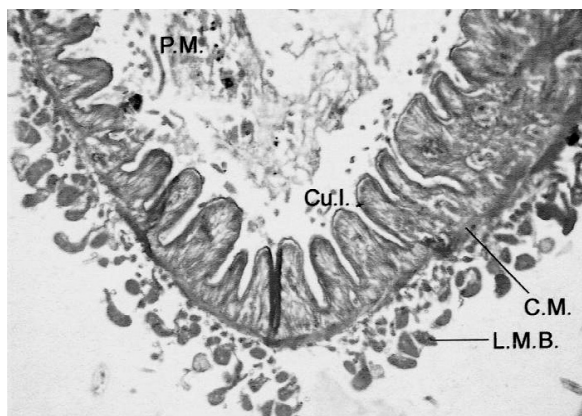


Figure 5: T. S. of alimentary canal passing through posterior region of midgut showing epithelial foldings and development of cuticular intima (H.E. x 100)

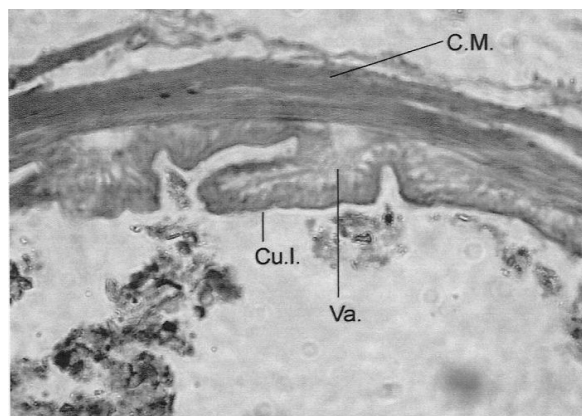


Figure 6: T. S. of alimentary canal passing through pyloric region, showing flat, pad-like epithelial folds and thick circular muscles (H.E. x 100)

can not easily be distinguished. In the middle region of the mid gut, the columnar epithelial cells are of uniform height.

The mid gut epithelium consists of two types of columnar epithelial cells (Fig. 4) long, cylindrical cells that are  $29\ \mu$  to  $44\ \mu$  in length, with basally located nuclei and short rounded cells, measuring about  $7.4\ \mu$  to  $14.8\ \mu$  in length. The short cells are found close to the basement membrane and wedged between the longer cells. The columnar cells are wider at the apical end. The surface of these cells presents a distinctive brush border of microvilli. Some of these cells show presence of globules which could be secretory material. In several sections, these epithelial cells are found in the process of fragmentation. The remains of these cells are also seen in the mid gut lumen. The secretion of mid gut cells seems to be of holocrine as well as merocrine type. The epithelial cells are stained dark purple with haematoxylin-eosin and their nuclei are more differentiated.

The posterior part of mid gut epithelium shows fold like structures and development of cuticular intima is also seen in some sections. The height of the epithelial cells is reduced in this region (Fig. 5).

At the posterior region, the circular muscles are also well developed and form a thick layer. Sheath layer is very much

reduced. Numerous longitudinal muscle bundles are seen outside the circular muscle layer.

**Pylorus:** At the junction of mid gut and hindgut, there is present a whitish, tubular structure called the pylorus. It is about 1.2 mm long and 1.5 mm wide. The epithelial lining of this region shows same pattern as that of the foregut except that the epithelial folds are more flat and wide, like the pads. Some vacuoles are also seen in the epithelial layer. Nucleus is present in the centre of the epithelial cells (Fig. 6).

The lumen of the pylorus is lined by a thick intima which is closely applied to the epithelial lining. Remains of the peritrophic membrane are also seen in some of the sections. Longitudinal muscle fibers are thin but more numerous. The circular muscle layer is very thick as compared to all other regions of the alimentary canal. A pair of Malpighian tubules opens at the anterior most region of the pylorus.

**Hindgut:** The hindgut is thin walled and relatively spacious region. The length of the hindgut is slightly more than that of the mid gut. The diameter of the hindgut varies with the amount of food material present in its lumen. Fig. 8 shows that the cuticular intima in the hindgut is thinner than that of the foregut. These cells are more uniform in size than these of the mid gut. The epithelial layer is folded irregularly but these folds are of lesser height. Basally, the epithelium is bounded by a thin basement membrane. The circular muscles are comparatively well developed. The longitudinal musculature consists of bundles of fibers distributed unevenly around the circular muscle layer. The sheath layer is absent in this region.

The hindgut in the millipede *Anoplodesmus tanjoricus* shows morphologically three distinct regions - a short anterior region called ileum; a long middle region called colon and a short, narrow, tubular region called the rectum.

i) **Ileum:** It is the anterior most part of the hindgut adjacent to the pylorus (Fig. 7). It measures 4.6 mm in length and about 1.8 mm wide. The epithelial cells in this region are short and cylindrical measuring about  $18\ \mu$  to  $22\ \mu$  in length and forms dense, branched folds. Longitudinal muscles bundles are present, circular muscles form a thin layer and are less conspicuous. In this region, cuticular intima is clearly seen which is placed very close to the epithelial lining. The epithelial cells of this region are short, cylindrical and are of equal heights.



Figure 7: T. S. of alimentary canal passing through ileum showing folded epithelium (H.E. x 400)

ii) Colon: The middle region of the hindgut is the longest region as compared to the other two regions of the hindgut (Fig. 8). It measures about 11.6 mm in length and 2.5 mm in width. The columnar epithelial cells are of same height. Intima is placed at a distance from the epithelial cell lining thus keeping the contents of the gut away from it. Nucleus is present in the middle region of the cell. The circular muscle layer is thin but clearly visible. The longitudinal muscle bundles are randomly arranged. Sheath layer is absent.

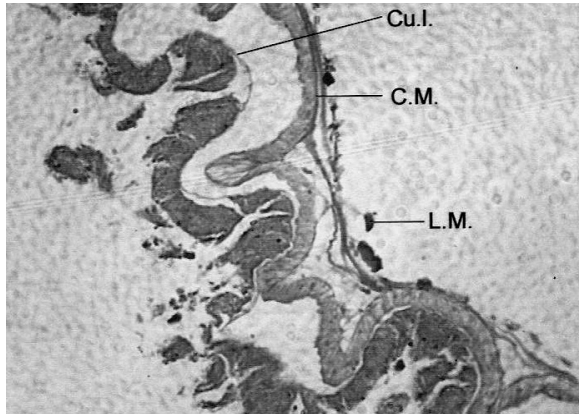


Figure 8: T. S. of alimentary canal through colon wall. The low foldings, uniform height of the epithelial cells and thin intima are seen (H.E. x 400)

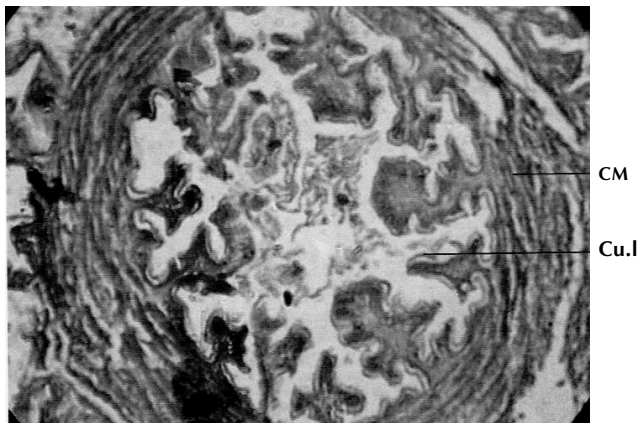


Figure 9: T. S. of rectum showing prominent circular muscles, thin intima and branched epithelial foldings (H.E. x 400)

iii) Rectum: The last part of the hindgut is the rectum, which forms a short, whitish, bulblike structure, measuring about 2.3 mm in length (Fig. 9). In this region, the cuticular intima is comparatively thin than the other two chambers of the hindgut and is closely applied to the epithelial lining. The epithelium forms six long branched folds forming papillae. Circular muscle layer is highly prominent about  $74\mu$  in thickness; however, the longitudinal musculature appears poorly developed. This may be helpful in eversion of rectum to imbibe moisture from the soil surface. The sheath is completely absent in this region of the hindgut.

## DISCUSSION

Cuticular intima lining, the foregut of *Anoplodesmus*

*tanjoricus* was also observed in other millipedes by Hefner (1929), Miley (1930), Nunez and Crawford (1977) and Shukla (1981). Posteriorly, the foregut projects into the lumen of the anterior midgut forming an oesophageal valve. Presence of oesophageal valve has also been reported by Nunez and Crawford (1977) and Shukla (1981). In this millipede, midgut is characterized by the presence of peritrophic membrane. The presence of peritrophic membrane was also recorded by Krishnan (1968), Nunez and Crawford (1977) and Shukla (1981). The sheath layer surrounding the midgut epithelium is highly developed, which may act as store house for glycogen (Bowen, 1968).

In the millipede *Anoplodesmus tanjoricus*, the posterior region of the midgut epithelium shows distinct, finger like folding and development of cuticular intima as also observed by Shukla (1981) in the millipede *T. lumbricinus*. A short tubular pyloric region separates the midgut from the hindgut. Epithelial layer in this region is lined with intima, epithelial folds are wider and flatter like pads and circular muscles are highly developed (Nunez and Crawford, 1977; Shukla, 1981).

Morphologically, hindgut is divided into three distinct regions – ileum, colon and rectum. Hindgut lumen is lined with cuticular intima, circular muscles form a thin sheath, longitudinal muscle bundles are randomly arranged and sheath layer is nearly absent (Nunez and Crawford, 1977; Shukla, 1981). Rectal epithelium forms six prominent folds called papillae, circular muscle layer is highly developed and longitudinal musculature is poorly developed but reaches the posterior end of rectum. The rectum shows ability of eversion which probably help to imbibe moisture from the soil surface. Sheath layer is completely absent (Nunez and Crawford, 1977; Shukla, 1981).

A pair of salivary gland is present from which arise numerous salivary ducts, which run forward and open at the bases of gnathochelarium (Nunez and Crawford, 1977; Shukla, 1981).

## REFERENCES

- Attem, C. G. 1936. Diplopoda of India. *Em. Ind. Mus.* **XI**: 133.
- Bowen, R. C. 1968. Histochemical studies on two millipede species. *Ohio. J. Sci.* **68**: 85-91.
- Hefner, R. A. 1929. Studies on Parajulid Diplopods No. 11. The microanatomy of the alimentary canal of *Parajulus impressus* say. *Trans. Amer. Micrsc. Soc.* **XLVIII**: 321-350.
- Krishnan 1968. The millipede *Thyropygus posseidon*, CSIR Zoological Memoirs on Indian Animal type no. 1. Publication and Information Directorate, New Delhi. p.80.
- Kumar, R. 1983. Studies on certain aspects of digestive physiology of the millipede *Gonoplectus malayas* (Carl). Ph. D. Thesis. Dr. Ram Manohar Lohia Avadha University, Faizabad.
- Miley, H. H. 1930. Internal anatomy of *Euryrus erythropygus* (Brandt) (Diplopoda). *Ohio. J. Sci.* **30**: 229-249.
- Nunez, F. S. and Crawford, C. S. 1977. Anatomy and histology of the alimentary tract of the desert millipede *Orthoporus ornatus* (Girard) (Diplopoda: Spirostreptidae). *J. Morph.* **151**: 121-130.
- Shukla, S. C. 1981. Histomorphology and physiology of alimentary canal of millipede *Trigoniulus lumbricinus* (Gerstaecker). Ph.D. Thesis. Univ. of Gorakhpur, Gorakhpur (India).