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Histomorphology of the larval hindgut of the Dragonfly *Bradinopyga geminata* (Rambur) (Odonata: Libellulidae)

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ABSTRACT

The larval hindgut of dragonfly *Bradinopyga geminata* (Rambur), was studied with light microscopy. The hindgut of dragonfly differentiate into ileum and rectum. The ileum is narrow tubular at anterior and posteriorly it is dilated spherical bulb. Ileum is located between midgut and rectum of hindgut. Ileum occupies the 20% length of total length of the alimentary canal. In the transverse section of ileum measure 430μm- 450μm diameter approximately. The internal 6 longitudinal folds are of unequal height and project into the lumen irregularly. The epithelium of ileum is consists single layer cuboidal epithelium with finely granular cytoplasm. The inner surface of the epithelium covered with by a prominent cuticle. The rectum is usually enlarged sac like structure which posteriorly connects with anal tube, the modified structure of anterior rectum in dragonfly is branchial chamber. It occupies the one third length of the alimentary canal. Rectum of dragonfly larvae is almost 3 times bigger in diameter than that of ileum. The rectum of larval dragonflies serves various functions such as swimming by jet-propulsion, breathing, storage of lipid and glycogen, and osmoregulatory salt uptake. Rectal basal pads encloses medulary fat cells from which pass trachea, number of gills projects into a rectal lumen and cover the maximum area of rectal lumen gills are underlying by thin cuticle.

Key words: Hindgut, Ileum, Rectum, Dragonfly, Larva

INTRODUCTION

The insect hindgut is located at posterior region of alimentary canal. Anteriorly it attached with midgut and posteriorly ends with anus. There is insufficient information available on the functional morphology and histological structure of hindgut of dragonfly *Bradinopyga geminata* (Rambur). However number of papers contributed significantly to certain aspects of the the hindgut, such as (Tillyard, 1917; Snodgrass,1954).

The hindgut isinternaly lined with cuticular layer which is thinner and permeable than foregut. The epithelium is thin, but the cells are more cuboid than in the foregut while those rectal pads are tall with clear cytoplasm, this includes pylorus, ileum and rectum (Chapman,1982; Tembhare and Wazalwar,2002). Both the foregut and hindgut have a cuticular lining, which is lacking in the midgut. The length of gut is roughly correlated with diet; insect feeding on a largely protein diet tends to have a shorter gut than those feeding largely on carbohydrates but this is not always true (Snodgrass,1935; Chapman,1982; Gullan and Cranston 2005). The anterior portion of the rectum has been modified into a branchial chamber with six gill folds it may be assumed that this part of the rectum is concerned with respiration as well as absorption of ions (Greven and Rudolph,1973). The ileum of anisopteran larvae reveals the existence of thick and thin epithelia. The thick epithelium apparently organised for ion transport. The cells are covered with a multilayered cuticula (Moens,1980). In terrestrial insects most of these ions and water are reabsorbed by the rectal papillae, because they live in fresh water, larvae of Odonata do not need to reabsorb water; however, the retention of salts is very important in osmoregulation. In these larvae the rectum is filled mainly with water, and thus reabsorption takes place in the first segment of the hindgut, the ileum (Moens, 1984). Dragonflies possess tracheal gills which are located in the rectum. Using stereological methods, we estimated the morphometric diffusing capacity for oxygen across the gill epithelium, i.e., from rectal water to the gill tracheoles, in the larvae of *Aeshna cyanea*. (Wichard and Komnick,1974; Kohnert *et al,* 2004). In Zygoptera, there are 3, in Anisoptera up to about 500 epithelial pads in the rectum which show fine structural features of transporting epithelia. Rectal ventilation provides contact of these epithelia to the external medium from where the ions are absorbed (**Neill** 1960; Hughes and Mill 1966; Mill and Pickard,1972; Komnick,1978; Miller,1994). The rectal chloride epithelia of the larval dragonfly *Aeshna cyanea*. These epithelia function in active ion absorption and maintain a high concentration gradient between the haemolymph and the fresh-water environment. (Schmitz and Komnick,1976; Kukulies and Komnick,1983). The larval rectum of dragonflies serves various functions such as swimming by jet-propulsion, breathing, storage of lipid and glycogen, and osmoregulatory salt uptake. All of them are more or less causally connected with rectal ventilation (Wichard and Komnick,1974; Pickard and Mill,1974; Mill and Pickard,1975; Komnick, 1982). The present work is therefore, proposed to understand functional morphology and histological structure of larval hindgut of dragonfly *Bradinopyga geminata* (Rambur).

METHODOLOGY

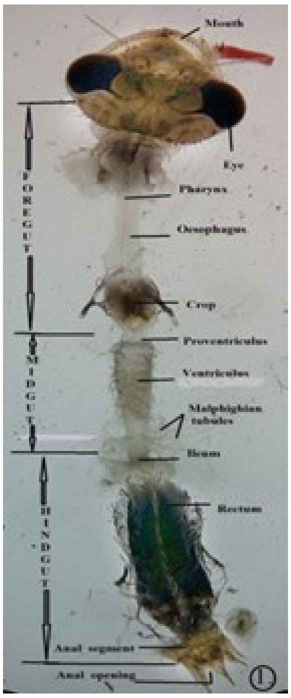
The dragonfly larvae collected from water tank with the help of nylon insect collecting net. The dissection was carried out in saline water under stereoscopic binocular microscope (ZEISS). Larval alimentary canal was exoposed and separate out the hindgut (ileum and rectum) form rest of the alimentary canal for further processing of morphological and histological demonstration. The hindgut especially ileum and rectum were fixed in Alcoholic Bouin's fixative for histological demonstration for a period of 12-18 hrs. The Bouin's fixed tissues were dehydrated in alcohol, cleared in xylene and embedded in paraffin wax at 60-62 ^{0}C. The sections of 4 - 6 µm thick were cut on Rotary microtome prepare slide and proceeded for histological H-E staining techniques. The slides were observed under microscope in 10x, 45x and 100x magnification and photographed.

RESULTS AND DISCUSSION

The wide variation in the insect alimentary structure has led to some inconsistencies in nomenclature. The terminology used in this paper follows that of Tillyard (1917) and Tembhare and Wazalwar(2002).

The alimentary canal of dragonfly *Bradinopyga geminata* (Rambur), consists of foregut divided into three parts pharynx, oesophagus and crop, midgut divided into two proventriculus and ventriculus and lastly hindgut divided into two anteriorly ileum and posteriorly rectum (Fig.1). In this paper our focus is on the morphology and histological structure of the hindgut these regions are considered separately as follows. Thelarval alimentary canal length approximately15 mm and total length of larva is near about 16-17mm.

Ileum: The ileum is tubular cylindrical structured organ lies behind malphighian tubules, anteriorly its narrow tube-like structure and posteriorly its dilated spherical bulb in shape (Fig.1). Ileum is S-shape, because of dorsally it attached at middle near the malphighian tubules. The ileum measures approximately 3mm in length it occupies the one fifth length of total length of the alimentary canal. In the transverse section of light microscopy ileum shows lateral diameter approximately 430µm - 450µm because of ileum is laterally compressed and dorsoventral diameter approximately 650 µm - 700 µm. The 6 longitudinal folds of ileal epithelium are of unequal height and project into the lumen irregularly (fig.2). The longitudinal muscle was weakly developed and the circular muscle are well developed, circular muscle in size approximately 3 µm (fig.3). The epithelium of ileum is consisting of a single layer of cuboidal epithelial cells are syncytial with finely granular cytoplasm, cell size approximately 4µm – 5 µm. Cell show deeply stained small nuclei without prominent nucleoli the nucleus size is 1 µm. The lumen surface of the epithelial cells is coated by a prominent cuticle (Fig.2,3&4).



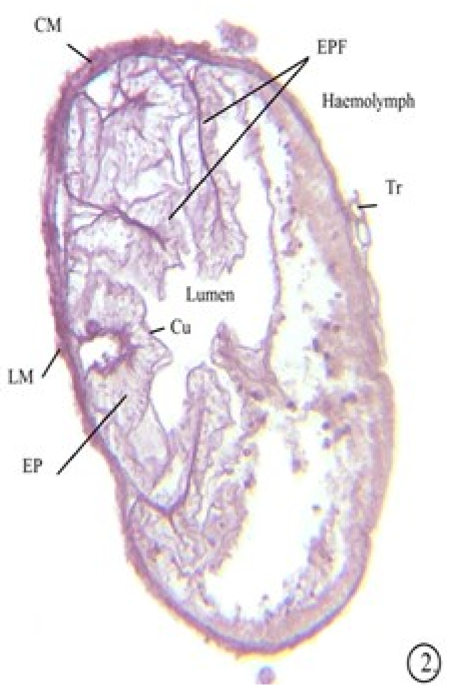
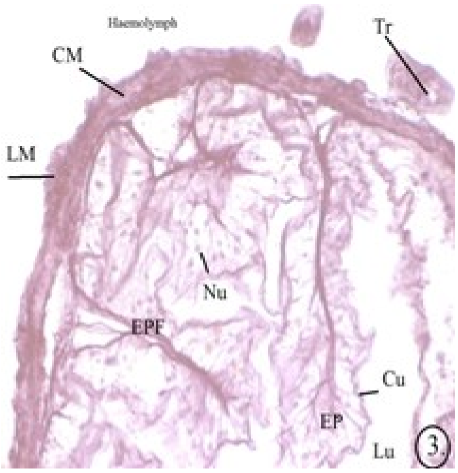


Fig.1- Whole mount of alimentary canal of larval dragonfly *Bradinopyga geminate* (Rambur), shows mouth, foregut, midgut, hindgut and anus.

Fig.2-Magnificatiion 100xCM- Circular muscle**, EPF-** Epithelial fold **,LM-** Longitudinal muscle**,EP-** Epithelial cells**,Cu-**Cuticle,**Tr-** Trachea, **Lu-**Lumen and **He-** Haemolymph.



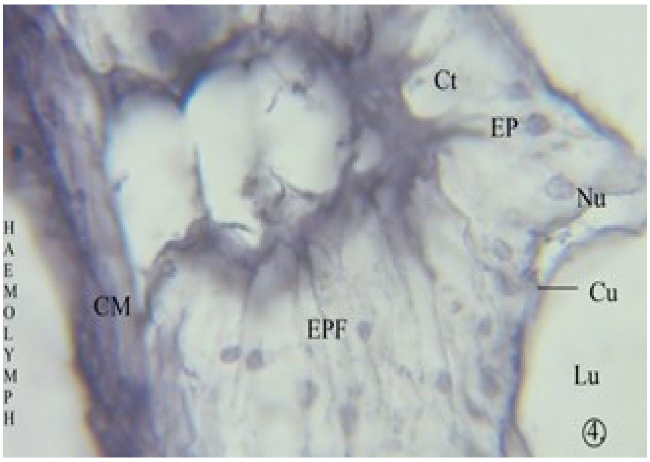
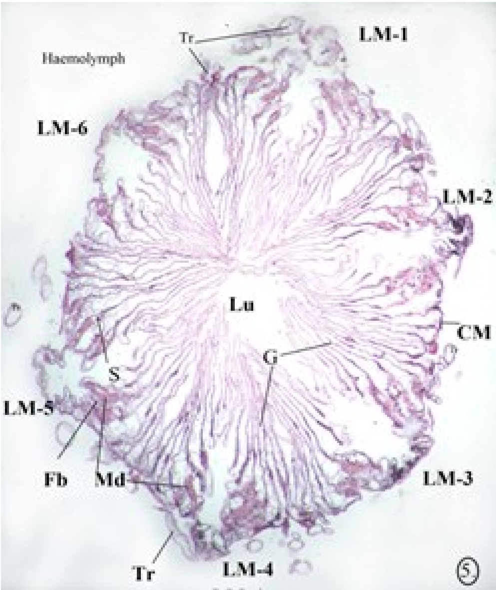


Fig.3- Magnificatiion 400xCM-Circular muscle, **LM-**Longitudinal muscle**, EPF-**Epithelial fold,**Nu-** Nucleus, **Cu-**Cuticle, **Ep-**Epithelial cells**, Lu-**Lumen,**Tr-**Trachea.

Fig.4- Magnificatiion 1000x CM-Circular muscle, **EPF-**Epithelial fold, **Ct-**Finely granular cytoplasm **EP-**Epithelial cells**, Nu-**Nucleus, **Cu-**Cuticle**, Lu-**Lumen.



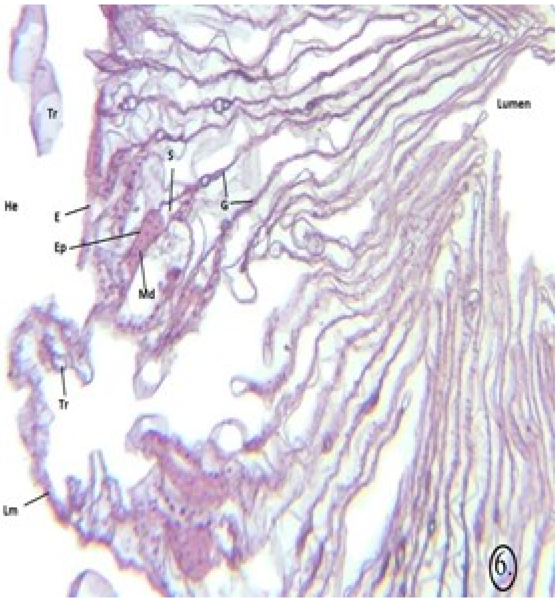


Fig.5-Magnificatiion 50x **LM-**6 Bands of lomgitudinal muscle**, Tr-**Trachea, **G-**Gill lamellae **CM-**Circular muscle,**Md-**Medullary cell **Fb-** Fat body cell **S-** sinus **Lu-**Lumen**, He-** Haemolymph.

Fig.6-Magnificatiion400x Tr-Trachea**, He-**Haemolymph**, E-**Epithelial lining of branchial chamber**,Ep-**Rectal pad Epithelium**, Md-** Medullary cell**, Lm-**Longitudinal muscle, **G-**Gill lamellae, **S-** sinus**,** lumen.

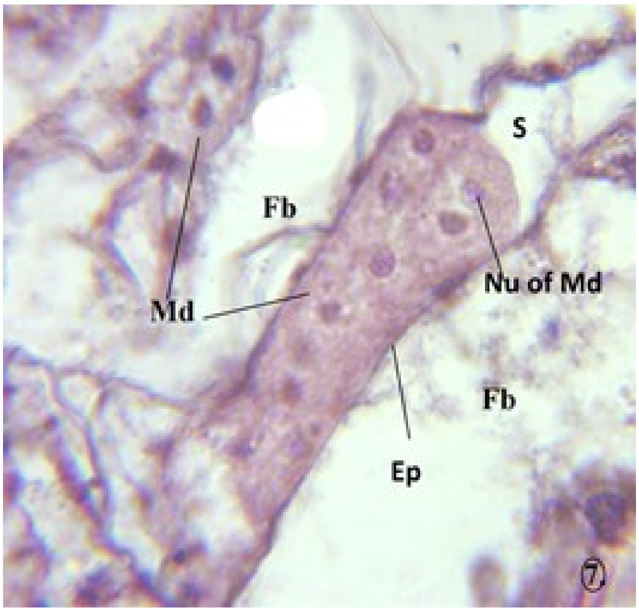


Fig.7-Magnificatiion1000x Md-medullary cells,**Fb-**Fat body cell**,Ep-**Rectal pad Epithelium**, Nu-**Nucleus**, S-**sinus

Rectum: The rectum is usually enlarged sac like structure which posteriorly connects with anal tube, the modified structure of anterior rectum in dragonfly is branchial chamber (Fig1). The rectum measures approximately 5 mm in length it occupies the one third length of the alimentary canal. In the transverse section rectum show lateral diameter approximately 1200µm - 1500µm because of rectum is laterally compressed and dorsoventral diameter approximately 1800µm-2000 µm. Rectum of dragonfly larvae is almost 3 times bigger in diameter than that of ileum, because the rectum of larval dragonflies serves various functions such as swimming by jet-propulsion, breathing, storage of lipid and glycogen, and osmoregulatory salt uptake. At the base of gill lamellae there are 6 pairs of basal pads with epithelial cells. Basal pad epithelial cell size approximately 3µm - 4µm and the nucleus size is about 1µm (Fig.7).

Rectal basal pads enclose medullary fat cells from which pass trachea (Fig.5 &6), number of gills projects into a rectal lumen and cover the maximum area of rectal lumen gills are underlying by thin cuticle (Fig.5 &6). The longitudinal muscle was well developed and having 6 rows cover entire length of rectum, because of these hexagonal structure shows and the circular muscle are also well developed (Fig.5). The pumping action of rectum during rectal respiration and jet-propulsionis for offence and defense mechanism toattack on pray or instantly go away from predator. There is a tremendous tracheal network covered the rectum and enter into a basal pad cell for rectal respiration by the help of rectal gills protruded in the lumen of the rectum 100 and above in number (Fig.5).

Alimentary canal of insects consists of three primary regions; foregut (stomodaeum), midgut (mesenteron), hindgut (proctodaeum). The gut epithelium is one cell-layer thick throughout the length of the canal. The gut is supported in body by muscle anteriorly and posteriorly, but elsewhere only by connective tissue and especially by tracheae which, in insects forms an important element of connective tissue. Both the foregut and hindgut have a cuticular lining, which is lacking in the midgut.The length of gut is roughly correlated with diet; insect feeding on a largely protein diet tend to have a shorter gut than those feeding largely on carbohydrates but this not always true(Snodgrass,1935; Cranston and Gullan, 2005).

In the hindgut of aphidophagous ladybird, *Adalia bipunctata*, the epithelium was cubical in the ileum and rectum and was squamous in the rectal canal. The ileum presented six longitudinal folds and a thin circular muscle layer (Borges, 2015).

The alimentary tract in the nymphs of dragonflies, mentioning the adaptation of the foregut for chewing and the hindgut for respiration and jet-propulsion. The dragonfly ileum turns upward in the fifth segment, close in front of the transverse muscle of the abdomen and expands into an oval sac. The intestine then continues as the huge respiratory chamber which arises by a narrow extremity from the upper end of the ileal sac and reaches to the end of the eighth abdominal segment. The respiratory chamber is commonly regarded as an enlarged anterior part of the rectum, but the narrow, cylindrical following part of the intestine has in itself the typical features of the rectum of other insects (Snodgrass, 1954).

The anterior hindgut is called the ileum, the generally narrower middle portion is the colon, and the expanded posterior section is the rectum. In many terrestrial insects the rectum is the only site of water and solute desorption from the excreta, the ileum makes some contribution to osmoregulation. The resorptive role of the rectum is indicated by its anatomy (Cranston and Gullan, 2005). In dragonfly *Bradinopyga geminate* the hindgut divided into ileum and rectum.

The numerous trachea found penetrating the thickened epithelium can be explained as necessary because respiration is more difficult in these regions owing to the thickness of the walls, hence the many tracheoles penetrating the thickened epithelium. A comparison with the rectal respiration areas of Anisoptera larvae seems to strengthen this view. In the rectal gills of these larvae, the minute branches of the tracheae are separated from the water of the rectum by a very thin epithelium. This seems to show that respiration takes place most actively through a thin epithelium (Cullen, 1918).

In Anisoptera, a pair of chitinous parts, which in life are constantly moving in a horizontal plane to and fro from each other in the process of respiration. The rectal tracheal-gills situated in the rectum probably function in constantly renew the water (Calvert, 1893).

In dragonfly long straight region of quite even diameter throughout most of segment seven the ileum, a decided bulbous enlargement throughout most of the eighth segment probably the pouched region of Carroll, a short constricted section entering segment nine and a very much thickened cylindrical region running through the ninth segment and most of the tenth. This enlargement has three broad folds along its whole length and between them thin, darkly pigmented areas; one of these folds is mid-dorsal, the other two latero-ventral, the anterior part of the rectum, the hind part of segment ten is occupied by a small vestibule leading to the anus, the walls of which are well supplied with tracheae (Whedon, 1918).

The relative importance of the role that respiratory, excretory, and cutaneous processes play is not as clear in aquatic insects, although three hypothesis may be considered. These hypotheses are (1) ion exchange across the cuticular respiratory surface is the dominant response to changes in extracellular acid-base balance in water breathing insects, (2) ventilatory control of hemolymph CO_2 is used for pH regulation by air breathing species, as observed in locusts and (3) differences in responses to pH changes in water and air breathing aquatic insects are only quantitative, not qualitative (Cooper,1994).

Well-developed rectal pads, it may be doubted whether the function of these pads is also absorption. It should, however, be remembered that the need for water conservation depends not only on the habit of the insect but also on the quantity of water in its food and the percentage of water in the body In aquatic forms, the pad is confined to the dorsal and lateral walls of the ileum and extends up to the junction of the ileum and rectum. The cells are tall, show long striations and rich tracheation (Bahadur, 1963).

The rich tracheal supply of the rectum of *Uropetala carovei* associated with the removal of dissolved oxygen from the medium, suggested that this tissue might be a suitable one for maintenance *in-vitro,* enabling the transport functions to be studied underideal experimental conditions. The present paper gives an account of experiments designed to establish that the rectum of *Uropetala carovei* was capable of active transport of ions under *in vitro* conditions (Green, 1978). The rectal lumen of anisopterous and zygopterous larvae is also ventilated through the anus fine structural and electrolyte histochemical results which suggest that the epithelial pads of zygopterous larvae are rectal chloride epithelia analogous to the rectal chloride epithelia of anisopterous larvae and probably osmoregulatory rather than respiratory in function (Komnick, 1974).

In anisopteran larvae light microscope, study epithelium lining the lumen of the ileum is composed of a thin and highly folded layer, and a thicker layer containing enlarged cells. Both epithelia are coated with a thin cuticula. The cells in the thin epithelium are nearly cuboidal, approximately 12 µm high, with a clear homogenous cytoplasm. Flattened nuclei which contain mosaic like dispersed chromatine occur in the basal parts of these cells. Most nuclei have two nucleoli. The thicker epithelium is composed of cylindrical cells which are spheric and spotted and occur more apically in the cell. An intensively stained zone is present just beneath the cuticula and in the basal part of these cells (Moens, 1980).

The tracheal gills in *Aeshna cyanea* are designed such that the tracheolar supply of the respiratory epithelia corresponds with the surface area of the gill lamellae. This is consistent with the hypothesis that rectal gills alone can satisfy the metabolic demand of the larvae. It would be of great interest whether the morphology of the branchial chamber can respond to hyperoxic or hypoxic environments during larval development. The ends of the gill lamellae from some approaches becomes clear, because the outer surface is relatively large and tracheoles appear to be particularly rare, diffusion distances could be extreme and these regions could have a special effect on the calculated diffusing capacity (Kohnert *et al*, 2004).

In *Libellula* gills the fat-body cells form an epithelium like stratum underneath the chloride epithelium. This stratum rests on a thin lining epithelium at the opposite side of the gill lamella (Komnick, 1982).

The respiratory epithelium of the rectal tracheal gills of *Aeshna cyanea* larvae is of cellular construction throughout the entire length. It contains numerous tracheoles surrounded by a thin cytoplasmic sheath of tracheoblasts and extracellularly located in deep invaginations of the epithelial cells. The tracheoles run nearly parallel to each other in radial orientation, they are, however, irregularly distributed with respect to their distance from each other and from the cuticle. This arrangement of the tracheoles presumably provides optimum conditions for oxygen uptake (Komnick,1974).

In most hymenopterans ileal lumen was lined by a cuticle that extended throughout its entire length and included the rectum. Transverse sections showed that the epithelium consisted of 4-6 folds that bulged into the lumen (Santos and Serrao,2006). In dragonfly *Bradinopyga geminata* the ileum transverse section shows 6 epithelial folds bulged in lumen.

In the larva of Palm Weevil, *Rhynchophorus phoenicis* the hindgut divided into ileum and rectum which terminates in the anus **(**Omotoso and Adedire,2010).The rectal sac is formed by enlargement of the rectal portion of the hindgut in *Bombyx mori*, it consists of epithelium, connective tissue, and many rectal pads, have a cortex and medulla(Izzetoglu and Ober, 2011).

In *Platynotus belli* epithelium of ileum consists of cuboidal cells supported by a prominent basement membrane. The nuclei of the epithelial cells are small & round. The intima is thin, chitinous and is provided with small spines. rectum is divided in to anterior rectum and posterior rectum. The epithelium of rectum bears broad folds and is made up of large cuboidal cells with large round nuclei. Internally, the epithelium is lined by thick intima and externally by isolated few longitudinal and well developed circular muscles. The longitudinal muscles are thrown into six external lengthwise bands give somewhat hexagonal appearance to the rectum (Sarwade and Bhawane,2013).

CONCLUSION

The alimentary canal of dragonfly *Bradinopyga geminata* (Rambur), consists of foregut, midgut and lastly hindgut divided into two ileum and rectum. The larval alimentary canal length approximately 15 mm and total length of larva is near about 16 - 17 mm. The ileum and rectum occupies the one fifth and one third length respectively of the alimentary canal. The rectum is usually enlarged sac like structure because the rectum of larval dragonflies serves various functions such as swimming by jet-propulsion, breathing, storage of lipid and glycogen, and osmoregulatory salt uptake. The longitudinal muscle are well developed and having 6 rows cover entire length, circular muscles are also well develop for pumping of rectum.

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