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Anatomical Features of the Gut System in Deudorix Isocrates Larval Stage (Coleoptera: Lycaenidae)

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ABSTRACT

This study provides the first detailed anatomical and histological examination of the alimentary canal in the final instar larva of *Deudorix isocrates* (Fab.), a topic previously unexplored. The alimentary canal is divided into three main sections: the foregut, the midgut, and the hindgut. The foregut consists of the buccal cavity, esophagus, pharynx, and crop. The buccal cavity, pharynx, and esophagus walls are lined with cuboidal epithelium, forming 6 longitudinal folds. The posterior pharynx is characterized by longitudinal ridges and backwardly directed bristles. The intima in this area is thin, with numerous folds of squamous epithelium, and the musculature is minimal. A stomodaeal valve with distinct histological features is present at the transition from the foregut. The midgut wall is made of simple columnar epithelium, containing scattered goblet cells and single regenerative cells, and is lined with a peritrophic membrane. The midgut also has thin musculature, consisting of an inner circular muscle layer and an outer longitudinal muscle layer. The hindgut, which includes the pylorus, colon, and rectum, is lined with cuboidal epithelium, while the ileum is lined with squamous epithelium. The intima in the hindgut is thinner than in the foregut, with patches of spines located near the posterior pylorus. The rectum is associated with the formation of the cryptonephridial system.

Keywords: Histology, Anatomy alimentary canal, *Deudorix isocrates*, Larva

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Introduction

The pomegranate fruit borer, *Deudorix Isocrates* (Fab.), is a major economic pest that damages both pomegranate and guava crops [1, 2]. It represents the primary obstacle to the production of pomegranates with sufficient quantity and quality. As a polyphagous pest, *D. isocrates* affects many plants such as citrus, pomegranate, guava, apple, litchi, peach, plum, pear, and sapota [3]. Extensive research has focused on its ecological behavior and control strategies to limit its impact on pomegranate yields. However, no significant anatomical or histological studies have been carried out on this pest, even though they are crucial for advancing pest management. The structure and function of the alimentary canal in arthropods are closely tied to how they digest and absorb their specific diets [4]. The midgut, being the largest portion of the alimentary canal, is vital for food digestion, nutrient absorption, and the processing of both chemical and biological insecticides [5-9]. Therefore, anatomical and histological investigations play a significant role in evaluating the effectiveness of insecticidal plant extracts [10-

13], understanding the impact of biological control agents [14], testing synthetic insecticides [15], and assessing the influence of various drugs [16] on lepidopteran pests. This growing interest in studying the anatomy and histology of the alimentary canal in insect pests has led to our research. This study provides the first comprehensive anatomical and histological description of the alimentary canal of *D. isocrates* larvae, offering essential data for toxicological and pathological investigations of this pest.

Materials and Methods

Specimens were collected through laboratory rearing and from infested pomegranate fruits in the field, where *D. isocrates* larvae were present. For anatomical examination, fresh larvae were euthanized by immersing them in hot water. The specimens were then dissected along the mid-dorsal line in insect saline, using a stereoscopic dissecting microscope. Morphometric measurements were taken with ocular and stage micrometers. The data were presented as averages with standard error (SE), as shown in **Table 1**.

Table 1. Morphometric measurements of the alimentary canal of *D. isocrates* larva.

Organs of the alimentary canal	Average width (± SE)	Average length (± SE)
Pharynx	0.631 (± 0.031)	0.742 (± 0.041)
Oesophagus	0.578 (± 0.014)	0.902 (± 0.056)
Crop	1.173 (± 0.065)	0.578 (± 0.022)
Midgut	3.976 (± 0.262)	16.780 (± 0.887)
Pylorus	1.810 (± 0.043)	2.020 (± 0.092)
Ileum	2.280 (± 0.128)	3.470 (± 0.247)
Rectum	2.280 (± 0.090)	2.800 (± 0.213)
Total	27.294 (± 1.105)	

Note: SE = Standard Error

Histological preparations were made from live specimens obtained from laboratory-reared stock. The specimens were dissected in a 0.7% saline solution [17]. The complete alimentary canal was divided into six sections: one section containing the foregut and a little part of the midgut, four sections from the midgut, and one that included the hindgut along with a small part of the midgut. Before fixation, the midgut's peritrophic membrane and its contents were carefully removed. The segments of the alimentary canal were then immediately placed in vials with different fixatives, including aqueous Bouin's, Carnoy's, formal saline, and chilled acetone. Bouin's was found to be the most suitable for the study and was therefore chosen for further histological work. The specimens were kept in Bouin's for twelve hours, washed for five to six hours under running tap water, and then dehydrated through increasing concentrations of alcohol. After clearing in benzene, the tissues were infiltrated with paraffin wax (M. P. 52–54 °C) for two and a half hours. Serial sections were made, both longitudinally and crosswise, at a thickness of 7 µm using a rotary microtome. These sections were placed on slides pre-coated with egg albumin [18]. Staining was done using Delafield's hematoxylin and 1 percent alcoholic eosin. Once stained, the sections were mounted in DPX after being cleared in xylene. Histological observations were performed under a light microscope at magnifications of 10X and 45X.

Results and Discussion

In *D. isocrates* larvae, the alimentary canal runs nearly straight and is almost equal in length to the body. It is divided into three main regions: the foregut (stomodaeum), midgut (ventriculus), and hindgut (proctodaeum). These sections are identifiable by distinct expansions and narrowing points along the canal. Histologically, the canal's structure consists of a simple epithelial lining, with surrounding longitudinal and circular muscle layers. The foregut and hindgut, both arising from the ectoderm, secrete a cuticular intimal layer that lines the alimentary canal [19].

The foregut is divided into four parts: the buccal cavity, pharynx, esophagus, and crop [20] **(Figure 1)**, a typical arrangement in lepidopteran larvae. The buccal cavity, located at the front, is histologically characterized by a thin intima, a delicate squamous epithelium, a layer of circular muscles, and longitudinal muscle bundles within the folds. Moving posteriorly, the pharynx is positioned just before the supra-oesophageal ganglia and leads into the esophagus, which is located after the ganglia. The pharynx itself is further divided into anterior and posterior regions. The anterior pharynx has a thicker intima compared to the buccal cavity, and its epithelial lining is made up of cuboidal cells with centrally located round nuclei. Six longitudinal folds are present, with a thick circular muscle layer surrounding the pharynx **(Figure 2)**. Longitudinal muscle bundles are found beneath the epithelial lining where the folds are wider. The posterior pharynx, in contrast, has a thick intimal lining forming overlapping ridges, with backward-facing bristles, while the cuboidal epithelium and circular muscle layer are similar to those in the anterior pharynx.

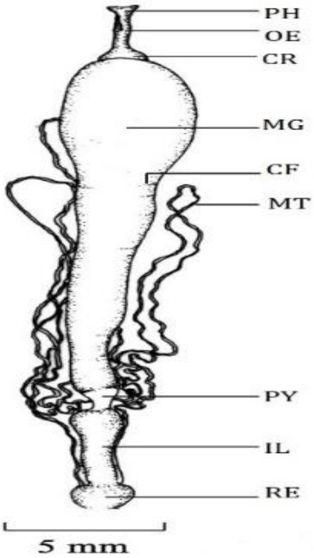

Figure 1. Alimentary canal of *D. isocrates* larva; CR: crop, IL: ileum, MG: midgut, MT: malpighian tubule, OE: esophagus, PH: pharynx, PY: pylorus, and RE: rectum.

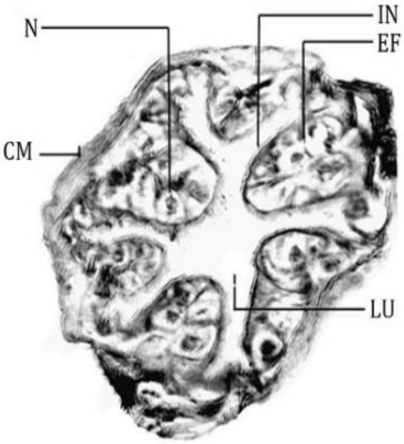

Figure 2. T.S. of the anterior pharynx, *D. isocrates* larva (x 113); CM: circular muscle, EF: epithelial fold, IN: intima, LU: lumen, and N: nucleus.

The pharynx leads directly into the esophagus, which forms a slender passage. The intimal lining of the esophagus is notably thinner compared to the pharynx and does not feature any ridges or bristles. The epithelial cells in this region are slightly larger cuboidal cells, with oval nuclei arranged closely together. This type of epithelium is also found in other species of lepidopterans, such as Heliothis virescens [21]. The circular muscle layer in the esophagus is relatively thin, while the longitudinal muscles extend through the epithelial folds, and the circular muscles form a continuous layer around the epithelial surface.

The esophagus extends into the crop, a sac-like region in the middle of the prothoracic segment, where it widens [22, 23]. Historically, this has been considered simply an expansion of the posterior esophagus [24]. The crop has thin, translucent walls and plays a crucial role in storing and directing ingested food [24, 25]. Its wall comprises a delicate intimal lining and deeply folded squamous epithelium **(Figure 3)**, which allows for significant expansion to accommodate ingested food [4]. In some areas, the muscle layer is difficult to discern from the epithelial base, a finding similar to observations made in the larvae of Lymantria dispar [4].

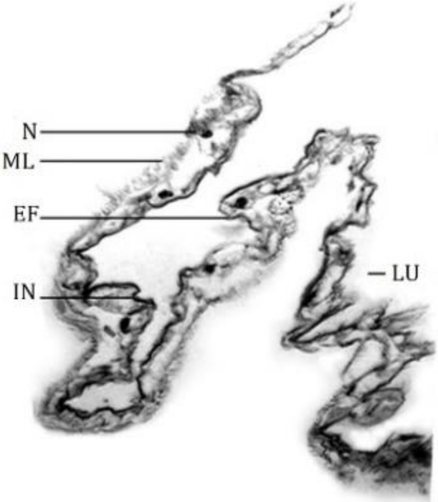

Figure 3. T.S. of the crop – in part, *D. isocrates* larva (x 125); EF: epithelial fold, IN: intima, LU: lumen, ML: muscle layer, and N: nucleus of squamous epithelial cell.

The transition from the crop to the midgut is marked by a distinct junction where the ventral side of the midgut extends more anteriorly than the dorsal side, creating an oblique angle. This causes the crop to be pushed towards the posterior region, effectively inserting its end into the lumen of the midgut. This internal protrusion forms the stomodaeal valve [22], or cardiac valve [4], which is analogous to the proventriculus [26].

After the stomodaeal valve begins, it doesn't form a complete cylindrical shape, as the posterior half extends further than the anterior half. The sides of the elongated region curve inward, forming a near cylindrical passage at the valve's posterior end. From a histological perspective, it consists of two distinct layers: an inner adaxial layer and an outer abaxial layer, similar to the observations in Helicoverpa armigera [23]. The adaxial layer, which is the inner layer, extends into the midgut for a short distance before it turns anteriorly, creating the abaxial layer. The two layers' epithelial linings are positioned closely but remain opposite each other. The intimal lining of the stomodaeal valve is thicker compared to the crop. Eventually, the abaxial layer merges with the midgut wall at the anterior interstitial ring, where the intima becomes notably thicker, forming a collar-like structure **(Figure 4)**. The interstitial cell lining is characterized by a thinner intimal layer, and the epithelial cells are simple columnar with oval-shaped nuclei. Regarding musculature, the foregut consists of both outer circular and inner longitudinal muscles [27], a common anatomical feature in the larvae of various lepidopteran species.

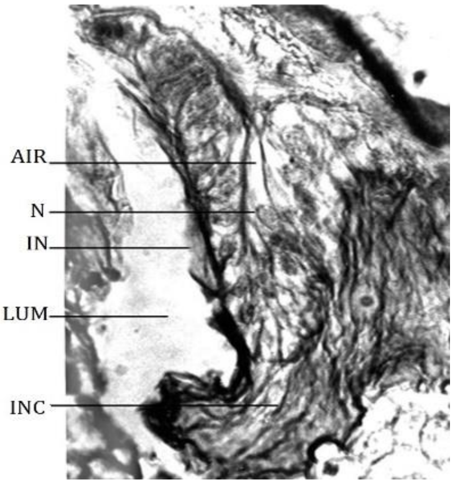

Figure 4. Magnified L.S. passing through the anterior interstitial ring, *D. isocrates* larva (x 443); AIR: anterior interstitial ring, INC: collar formed by thick intima, IN: intima, LUM: lumen of the midgut, and N: nucleus of columnar epithelial cell.

The midgut is the longest section of the alimentary canal **(Figure 1)**, extending from the mesothoracic segment to the fourth abdominal segment. It is held in place by tracheal attachments. The anterior portion of the midgut is more dilated compared to the posterior portion. The midgut wall is thicker than that of both the foregut and hindgut. When empty, the wall exhibits transverse folds, which are commonly observed in the digestive systems of lepidopteran species. These folds contribute to the elasticity of the wall, allowing the gut to accommodate a large volume of food [21]. Small, bud-like structures known as gastric caeca are present along the midgut, though they are not externally visible due to the folds in the wall. The midgut is composed of the peritrophic membrane, epithelial layers, and muscle layers. The peritrophic membrane serves as a protective barrier, preventing food particles from damaging the microvilli of the epithelial cells [28]. In this research, the peritrophic membrane was removed along with the gut contents to ensure proper fixation, which is why it is absent in the sections analyzed.
The midgut epithelium **(Figure 5)** is made up of simple columnar cells, along with regenerative and goblet cells, similar to those found in other lepidopteran larvae [29-31]. The columnar cells are tall, with tightly packed ovoid or round nuclei, located either at the apical or misal positions. Regenerative cells are scattered individually at the base of the epithelium, along the basement membrane. This distribution differs from that observed in Lymantria dispar larvae [4] but is consistent with what is seen in *H. cecropia* larvae [19]. This arrangement of regenerative cells is characteristic of continuous feeders. These cells play a crucial role in epithelial renewal and support gut growth during the process of ecdysis [32-34]. Mesenteric caeca are found throughout the length of the midgut, with an epithelium similar to that of the midgut itself. The circular muscle layer in the midgut is thin, with bundles of longitudinal muscles interspersed around it.

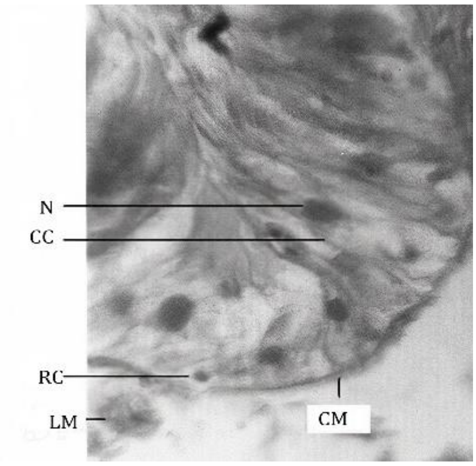

Figure 5. Magnified part, T.S. of the midgut, *D. isocrates* larva (x 375); CC: columnar epithelial cell, CM: circular muscle, LM: longitudinal muscle, N: nucleus, and RC: regenerative cell.

In *D. isocrates* larvae, the hindgut consists of several segments: the pylorus, colon, ileum, and rectum **(Figure 1)**, which is consistent with what is observed in other lepidopteran species [7, 19, 21, 35]. Anatomically, the hindgut begins at the posterior interstitial ring, characterized by a thick lining. The epithelium of this region extends from the posterior end of the midgut and is composed of simple columnar cells with oval nuclei, resembling *Heliothis virescens* [21]. In some species, like *Heliothis zea* and *Anticarsia gemmatalis*, the epithelium of the posterior interstitial ring is formed by cuboidal cells [7, 36], and similarly to the anterior interstitial ring, it shows a weak affinity for common staining methods like eosin and hematoxylin.
The pylorus, situated in the fifth abdominal segment, projects forward from the rear end of the midgut. The pyloric wall is thinner compared to the midgut and lacks the transverse folds seen in the latter. The anterior section of the pylorus is broader, narrowing toward the rear where it forms a tube-like structure. In essence, the pylorus is funnel-shaped. The lateral sides of the pylorus are connected to ducts that branch into three distinct segments, which is a unique branching pattern. From the common duct, two primary branches form; one of them bifurcates, and the other stays unbranched. These three branches extend to form the *Malpighian tubules*, which run anteriorly along

the midgut wall until they curve backward toward the rectum, forming a cryptonephridial arrangement. A similar pattern has been noted in *Heliothis virescens* larvae [21].

Histologically, the pylorus is divided into three regions: the pyloric valve, anterior pylorus, and posterior pylorus. The pyloric valve is formed by the posterior interstitial ring and the anterior part of the pylorus, characterized by inwardly folded structures. The intima of the pyloric valve is notably thick, and the cuboidal epithelium in this region consists of large cuboidal cells and smaller scattered cuboidal cells. The anterior pylorus features a thinner intima compared to the pyloric valve, with larger cuboidal cells and large, round nuclei positioned centrally. The folds of the epithelium in this section are irregular. In contrast, the posterior pylorus shows distinct features, including areas covered with sclerotized spines **(Figure 6)**, which are believed to aid in breaking off the peritrophic membrane as it moves from the midgut [37] and may assist in fecal propulsion [4]. The epithelial structure in the posterior pylorus consists of large cuboidal cells with round nuclei, and it forms irregular, prominent folds that lead to a thickened wall. These folds constrict the lumen where the pylorus connects to the ileum. At this junction, the epithelium shifts to longitudinal folds that merge with similar folds in the ileum. A circular muscle layer surrounds the epithelium, with longitudinal muscle bundles positioned beneath and close to the epithelial base.

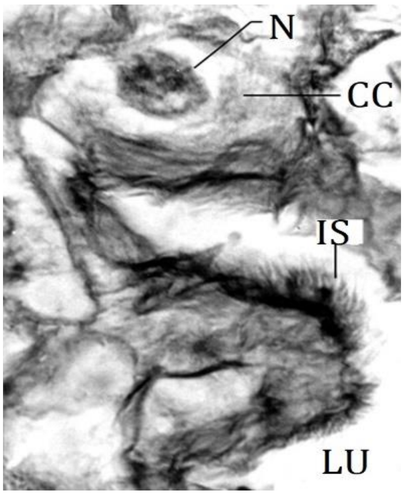

Figure 6. Magnified part of epithelial fold from posterior pylorus *D. isocrates* larva (x 540); CC: cuboidal epithelial cell, LU: lumen, N: nucleus, and IS: patch of intimal spines.

The pylorus connects to the ileum, which represents the longest section of the hindgut, located in the 6th and 7th abdominal segments. The ileum is wider in its anterior portion, gradually narrowing as it extends posteriorly. Its lining is characterized by wavy intima. The epithelial layer consists of squamous cells, similar to those observed in *Heliothis virescens* [21], with spherical nuclei. The epithelium is arranged in numerous longitudinal folds, and beneath it lies a muscle layer consisting of a thick inner circular muscle and outer longitudinal muscle bundles.

The ileum transitions into the rectum, passing through the colon, which is not distinctly visible externally, a feature common to other Lepidoptera species like *Heliothis virescens* [21]. The intima of the colon also appears wavy, and its epithelial cells are cuboidal with spherical nuclei, located either misally or basally. This region's epithelium forms complex folds that occlude the lumen. The muscle layer in the colon is thicker than that of the ileum, containing both an inner thick circular muscle layer and an outer longitudinal muscle layer.

The rectum, a roughly round sac, has a thin, translucent wall and opens externally via the anal opening located on the ventral side of the 10th abdominal segment. The rectal wall is pierced by the Malpighian tubules, exhibiting a cryptonephridial arrangement typical for Lepidoptera larvae [38]. The rectal intima is thin, and the epithelium is made up of large cuboidal cells with round nuclei. The epithelium forms irregular folds of varying sizes. The muscle structure of the rectum includes both a thick circular muscle layer on the inside and longitudinal muscle bundles on the outside.

Conclusion

In the larva of *D. isocrates*, the alimentary canal, which includes a distinct midgut, foregut, and hindgut, closely matches the body's length. From a histological standpoint, the alimentary canal's wall consists of a simple epithelial layer, surrounded by longitudinal muscle bundles and a circular muscle layer. While the foregut and hindgut are lined with a cuticular intima, the midgut features a peritrophic membrane. Notably, the foregut's intima is thicker compared to that of the hindgut. The foregut is subdivided into the pharynx, esophagus, buccal cavity, and crop. Within the crop, the stomodaeal valve extends into the midgut lumen. The posterior pharynx's intima has characteristic overlapping ridges and backwardly directed bristles, while the esophagus, buccal cavity, and crop have smooth and thin intima. The crop contains a thin, extensively folded squamous epithelium, whereas other foregut regions show a cuboidal epithelium with six longitudinal folds. Circular muscles surround the longitudinal muscle bundles and epithelium and extend through the bases of epithelial folds up to the esophagus. The crop's musculature is thin and covers the epithelium externally.

The midgut, the longest section of the alimentary canal, features a peritrophic membrane, simple columnar epithelium with goblet cells, and regenerative cells arranged near the basement membrane. The gastric caeca shares similar histological characteristics with the midgut. The midgut is bordered by anterior and posterior interstitial rings, composed of small columnar epithelium. The hindgut is made up of the pylorus, ileum, colon, and rectum, with a cryptonephridial excretory system. The hindgut's intima is thinner than that of the foregut and contains sclerotized spines at the posterior pylorus. The pyloric epithelium is cuboidal and forms prominent longitudinal folds that occlude the lumen. The ileum's squamous epithelium is arranged in numerous longitudinal folds, with a wavy intima. The muscle layer of the ileum includes a thick inner circular muscle layer and outer longitudinal muscles. The colon has cuboidal epithelium arranged in complex folds, and the rectum's cuboidal epithelium forms irregular, variously shaped folds. The rectal musculature comprises thick circular and longitudinal muscle layers.

This study offers a thorough description of the anatomy and histology of the alimentary canal, which will be valuable for future research into the toxicity, physiology, and pathogenicity of both herbal and synthetic insecticides. The insights gained can aid in developing effective pest management strategies for controlling this pest.

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