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MORPHOLOGICAL STUDIES ON THE FRESHWATER SNAIL *CLEOPATRA BULIMOIDES* (OLIVIER) (PROSOBRANCHIATA - PALUDOMIDAE). 1- THE DIGESTIVE SYSTEM

Magdy T. Khalil and Reda H. Aly.
Department of Zoology, Faculty of science, Ain Shams University.

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ABSTRACT

The detailed macro- and microscopic anatomy of the digestive system of the gastropod *Cleopatra bulimoides* has been yet described for the first time, and the results have been discussed with previous works on other related snails. The digestive system of the snail conforms with the basic plan of organization described for other members of the Cerithioidea. Particular stress has been laid upon specific or generic differences, if any, concerning the structure of the buccal mass, oesophagus, stomach, intestine, rectum and digestive glands.

INTRODUCTION

A review of the literature reveals that studies on the anatomy of the Cerithiacea in general and the Paludomidae in particular are very meagre. Scattered minor records are found in the works of Seshiaya (1929), on the structure of the stomach and the style sac in *Paludomus tanschaurica* (Gmelin), and of Johansson (1953 and 1956), on the pallial oviduct of some mesogastropods. Apart from the works of Demian *et al.* (1963) on *Pirenella conica;* Aboul-Ela and Beddeny (1969) on *Lanistes boltini* ; Yousif (1973) on *Melanoides tuberculatus;* Martoja *et al.* (1975) on the histology of the digestive system and digestion of the Atlantidae; Houbrick (1980 and 1981) on *Modulus modulus, Diastoma melanoides* (Reeve), *Campanile symbolicum* and *Gormya gormyi,* hardly any work has been done on other prosobranchs in Egypt.

Cleopatra snails have long aroused the interest of parasitologists since they were reported to act as intermediate hosts of the Equidae. However, apart from the original description of the shells, and a few scanty notes on the radulae of these snails, particularly nothing is so far known of their macro- and microscopic anatomy. Therefore, the detailed anatomy and histology of the digestive system of *Cleopatra bulimoides*

(Olivier) is herein considered as the first of a forthcoming series of studies dealing with the macro- and microscopic anatomy of the snail.

MATERIAL AND METHODS

Numerous adult snails were collected from irrigation canals in Giza Governerate. The specimens to be studied were placed in the lab, in plastic bowls containing dechlorinated tap water. For macroscopical studies, the snails were relaxed by adding few menthol crystals. Gross dissection was done under a dissecting microscope in waxed dishes. For histological studies, the snails were fixed either in Bouin, 10% formalin, or Zenker formol. The soft parts were detached from the shell and the digestive system was carefully separated in the fixative, dehydrated, cleared and embedded in paraffin. Sections were cut at 5-6 µ. thick and stained with Haematoxylin-Eosin. They were examined and photographed with a compound microscope built in camera lucida.

RESULTS AND DISCUSSION

The digestive system of *Cleopatra bulimoides* is composed of many organs namely, buccal mass, oesophagus, stomach, intestine, rectum, salivary and digestive glands (Fig. 1).

The buccal mass (Fig. 2)

A detailed analysis of its structure is given under the following separate parts:

The peristome (Fig. 4)

The mouth opening is surrounded by the peristome which is subdivided into two lateral lips. The margins around the opening are yellowish in colour with green and dark pigmented spots. Their lining epithelium is formed of tall narrow, columnar cells covered by a very thin cuticular layer.

The buccal vestibule (Fig. 2)

The folds marked on the peristome continue backwards forming the buccal vestibule which is lined by a chitinogenic epithelium and is devoid of mucous-secreting elements. It is made up of narrow columnar cells.

The mandibular region and jaws (Fig. 5)

The mouth opening is supported by a pair of chitinous jaws that projects from the buccal vestibule. Each jaw takes an oval outline, and shows minute serrations on the anterior edge.

The epithelial lining of the mandibular region is composed of normal chitinogenic columnar cells that are responsible for the secretion of the jaws. A narrow zone of delicate microvilli intervenes between the apical cell membranes and the overlying jaw material.

Two distinct layers can be histologically differentiated in the substance of each jaw which are found adjacent to the epithelial columnar layer: outer narrow columns and inner thin, compact, more flexible lamellae extending parallel to the surface of the jaw.

In Haematoxylin-Eosin preparations, the inner layer assumes a pink colour while the outer cuticular a faint yellow colour (Fig. 5). In Mallory's triple stained preparations, the inner layer acquires an orange-red colour, and the outer one assumes a light blue colour. This supports the work of Lutfy and Demian (1967) on *Marisa carnuarietis* and that of Sleem (1986) on *Bellamya uniclor*. However, in pulmonates, such as *Bulinus truncatus* (Haroun, 1970) and *Lymnaea caillaudi* (Mousa *et al.*, 1982), the jaw is formed of one cuticular layer.

The odontophoral region (Figs. 6 & 7)

Just behind the level of the jaws, the floor of the buccal cavity is raised into a large spheroidal prominence forming the odontophore. Dorsally, it carries the radular ribbon and the two lateral alary processes of the radula and the subradular organ, projecting from its anterior surface. The epithelium lining the anterior surface of the odontophoral mass, above the subradular organ, is made up of tall columnar chitinogenic cell- layer, devoid of glandular cells. Two long prominent dorsal buccal ridges hang down from the roof of the buccal cavity on the odontophoral region, one on each side of the median line. These enclose between them a long dorsal food channel, starting anteriorly between the two jaws and extending posteriorly to the oesophagus. This channel is lined with ciliated columnar epithelium, with mucous secreting cells.

Each dorsal buccal ridge is differentiated into two regions, an outer one which is highly glandular and less folded, and an inner one which is ciliated, with a few number of mucous secreting cells (Fig. 6).

The present study reveals the presence of two pairs of odntophoral cartilages in the buccal mass, namely the lateral and superior pairs. These cartilages serve both as a support for the radula and as a seat for the attachment of an elaborate system of extrinsic and intrinsic buccal muscles, that move and actuate the buccal mass and radula.

Each cartilage is nearly triangular in outline, with a dorsally directed tapering apex, while the other two angles are rounded.

Histologically the odontophoral cartilage is composed of cartilage cells (polygonal or irregular outline) with muscle fibres and vascular connective tissue in the matrix. Similar structure was reported in other prosobranchs (Prashad, 1925; Demian, 1954; Lutfy and Demian, 1964b and Sleem, 1986). In pulmonate snails, however, this is represented only by one pair of lateral cartilage (Pan, 1958, and Mousa *et al.*, 1982).

The radular sac and radula (Figs. 6 & 7)

The radular sac arises as a median, bilaterally compressed, evagination of the buccal cavity epithelium that goes deeply downwards in the odontophoral mass, behind the cartilages. This evagination has its lateral portions turned upwards so that it assumes the form of a double-layered incomplete cylinder. The two epithelial layers, forming the wall of this cylinder, enclose inbetween them the actual cavity of the evagination which thus appears highly compressed and crescent-shaped in cross-section. In this cavity, the nascent portion of the radula is found, and the two columnar epithelial layers in the wall of the cylinder contribute to its formation. The outer layer, lying below the radula, is referred to as the subradular epithelium, while the inner layer, found above the radula is the supraradular epithelium. The central region of the cylinder is filled up with a pale yellowish firm central core of some supportive tissue of irregular cells with rounded nuclei, forming what is known as the radular collostyle. Two broad semicircular , dentate membranes are present on both sides of the radula and are known as the alary processes. These are very thin, with a smooth convex upper surface.

The oesophagus (Figs. 1,2 & 8)

The oesophagus starts at the posterodorsal edge of the wall of the buccal mass, as a simple long, narrow tube which passes through the nerve ring (Figs. 1&2) and opens into the ventro-lateral side of the stomach. Anatomically, it can be differentiated into three regions; a short slightly compressed pro-oesophagus, a mid-oesophagus and a post-oesophagus that leads to the complex stomach.

Histologically, the oesophagus (Fig. 8) is lined with a layer of columnar ciliated cells with some goblet cells followed by a thick layer of a longitudinal muscle layer then a circular muscle layer. They are ensheathed by connective tissue. The pro-oesophagus has a pronounced dorsal food channel. As in many prosobranchs, the oesophagus of *C. bulimoides* does not show distinct oesophageal pouches.

The stomach (Figs. 1,3,9,10 & 11)

The stomach of *C. bulimoides* is an elongated biglobular chamber (Fig.1). It is generally divisible, by a constriction, into a large globular proximal posterior gastric portion, which receives the post-oesophagus and the digestive gland duct; and a smaller distal anterior pyloric portion, namely the style sac, (blind diverticulum) (Fig. 3).

The gastric portion appears bluish green in colour, in freshly preserved specimens while the sorting area, acquires a pigmented dark tinge. The oesophagus opens on its left ventro-lateral side and the two main hepatic ducts unite to form a wide hepatic vestibule (Fig. 9), which opens on the ventral side, behind the level of the oesphageal opening.

If a longitudinal dorso-lateral incision (Fig. 3) is made through the wall of the stomach, slightly to the right, a complex system of ciliated internal ventral folds is observed, projecting on the ventral walls of the two regions. There are three longitudinal folds, on the ventral side of the gastric portion, namely: the right, middle and left ventral folds. Similar structures have been described in the stomach of other prosobranchs under different terms. Seshaiya (1929), termed these folds as the central, marginal and outer ventral folds; Johansson (1956), named them as right, left and middle folds and Binder (1959), referred to them as principal, marginal and semicircular folds. Demian *et al.* (1963), and Yousif (1973), as well as the present writers adopted the terms previously applied by Johansson (1956).

According to Dazo (1965), the function of these folds is to direct the food contents within the stomach, and to aid in their thorough mixing.

A transparent flattened gastric shield "gastric portion", almost triangular membrane, with bent ends is situated opposite to the free end of the crystalline style (Fig. [illegible]).

The dorsal wall of the gastric portion of the stomach is provided with a large number of transverse ridges on the inner surface, called the sorting area. The part of the sorting area covering the wall of the gastric shield has a distinct black pigment. The fold of the gastric shield, is lined with tall columnar cells, which secrete a thick cuticular layer. The wall of the sorting area is lined with highly folded ciliated columnar epithelial layer provided with secretory goblet cells (Fig. 10).

The distal portion (Fig. 3) of the stomach is a blind diverticulum which leads to the style sac. This sac encloses the crystalline style (rod like) which is a translucent and flexible structure. Its rotation against the gastric shield helps in withdrawal and grinding the food particles. In the style sac there is a mid-ventral ciliated furrow, which runs through the greater length of the sac. The style sac is lined with brush-border tall columnar epithelium, provided with well developed uniform, long heavy coat of cilia. The function of the style was first indicated by Van der Schalie and Dundee (1956) in amnicolid snails. They observed that the style rotation helps to draw food into the stomach, and then served to grind it into smaller particles. According to Dazo (1965), the crystalline style of *Oncomelania* contains cellulase enzyme.

Both crystalline style and gastric shield have been described in many prosobranchs, by Seshaiya (1929), in *Paludomus tanschaurica;* Demian *et al.* (1963), in *Pirenella conica;* Starmuhlner (1969), in *Cleopatra coulbeaui;* Yousif (1973), in *Melanoides tuberculatus* and Houbrick (1980 a & b and 1981a) in *Modulus modulus.* Other forms as *Gourmya qourmyi* and *Campanile symbolicum* have only a gastric shield (Houbrick, 1980b and 1981a). According to Houbrick (1981b) and Sleem (1986), the cuticularized area in the stomach of some prosobranchs represent a primitive stage in the evolution of the gizzard found in pulmonates.

The intestine (Figs. 1, 3 & 12)

In *C. bulimoides*, the style sac leads directly to the intestine. The latter is a long coiled narrow tube, and lies mostly around the style sac, covered with the kidney and the heart from the dorsal side. Finally, it passes anteriorly to the rectum which opens by the anus at the mantle collar (Figs 1&8).

Anatomically, the intestine can hardly be differentiated externally into different parts. Histologically it can be divided into pro- and post-intestine. The pro-intestine, is characterized by the presence of a typhlosole, which runs dorsally along the whole of its length, but the rest of its wall is slightly folded. The typhlosole diminishes gradually in height distally to fade completely in the wall, before the beginning of the post-intestine.

The lining epithelium of the intestine is made up of ciliated columnar pseudostratified cells. The cilia are compactly arranged to form a distinct brush border. Mucous cells of the goblet type are scattered in the mucosa (Fig. 12). The above mentioned intestinal characters conform, at least morphologically, with what has previously been reported by Lutfy and Demian (1967), on *Marisa cornuarietis* and by Sleem (1986), on *Bellamya unicolor*.

The rectum (Fig. 1)

The rectum appears as a compressed tube. It can be differentiated externally from the intestine by its relatively larger diameter, and its somewhat darker colour due to its faecal content.

Histologically, the wall of the rectum shows prominent internal folds surrounding an irregular lumen and made up of low columnar cells with few mucous cells scattered inbetween.

The digestive glands (Figs. 1,9,12 & 13)

The glandular structures associated with the alimentary tract of prosobranch gastropods are generally three types, namely: the salivary glands, opening in the buccal cavity; the gastric or digestive glands, leading to the stomach, and the lateral oesophageal pouches or homologous structures. While the first two are almost of universal occurrence, the third may not be represented at all. The reason for the lack of the oesophageal pouches is bound up with the characteristics of style bearing animals i.e being continuous feeders without free protease in their guts (Younge, 1932).

Accordingly, in *C. bulimides,* the digestive glands are represented by one pair of salivary glands and the digestive gland or hepatopancreas.

The salivary glands (Figs. 1 & 12)

The salivary glands are branched tubular structures, which lie on either side of the pro-oesophagus, behind the nerve ring. The salivary glands discharge by two elongated ducts, one on each side and open into the latero-dorsal side of the buccal cavity, above the odontophoral mass (Fig. 1). The wall of the tubules are attached together by thin strands of connective tissue, and are lined by a single layer of ciliated columnar epithelial cells, in different stages of secretory activity (Fig. 12).

The primary function of the salivary glands appears to be the production of mucus for lubrication of the radula, and cementing together the food particles collected by it. In carnivores, the secretion of protease, and in herbivores various enzymes are produced which attack carbohydrates (Graham, 1939).

The digestive gland (Hepatopancreas) (Figs. 1,9 & 13)

The digestive gland is a distinctly large, brown, spirally coiled structure, lodged in the posterior part of the shell spire. The major part of the gland lies behind the stomach (Fig. 1). It is of the compound tubular type. The tubules are bound together by loose connective tissue. The digestive gland ducts open into the gastric portion of the stomach through a common hepatic vestibule (Fig. 9). The lining epithelium of the tubules, is composed of two main types of cells; the digestive and secretory cells.

The digestive cells (Fig. 13) are tall and columnar, with rounded apices and flat basal parts, showing different cyclic phases of activity. In the first stage, the cells are tall columnar, with basophilic granules and basal nuclei. The second is characterized by the presence of numerous small vesicles of various sizes, arranged near the apex. In the third stage, the cells attain maximum size in which the cytoplasm becomes confined to a narrow band and the major part is occupied by large vacuoles, containing small, yellowish spherules, which are released into the lumina of the digestive tubules after the rupture of the apical cell membranes.

The secretory cells are pyramidal and represented by smaller number than the digestive cells. The apices of these cells absorb the excretory materials excreted by the digestive cells, and in turn release them through their basal portions which bulge very close to one of the blood sinuses, lying in the surrounding connective tissue.

In the lining epithelium of the digestive tubules some undifferentiated columnar cells are often found. It has been assumed that such cells can be transformed into any of the two cell types of the digestive tubules, when there is such need. The wall of the digestive gland ducts is made up of ciliated cuboidal cells with rounded basal nuclei (Fig. 9).

According to Graham (1939), the function of these glands may be slightly modified in such prosobranchs which possess a crystalline style. The digestive and secretory cells of the digestive gland may be used not only for elaboration of enzymes, but also for absorption of food, storage, and excretion. This seems to support the observations made on other prosobranch snails by Graham (1932); Fretter and Graham (1962); and Lutfy and Demian (1967). In fact, the digestive cells in the present work correspond to the absorbing cells, the hepatic cells in the digestive glands of other snails (Graham, 1932; Michelson, 1955; Fretter and Graham, 1962). The secretory cells correspond to the ferment cells, the lime cells, the calcium secreting cells, or the excretory cells of Prashad (1925); Carriker and Bilstad (1946); Cleland (1954); Fretter and Graham (1962) respectively.

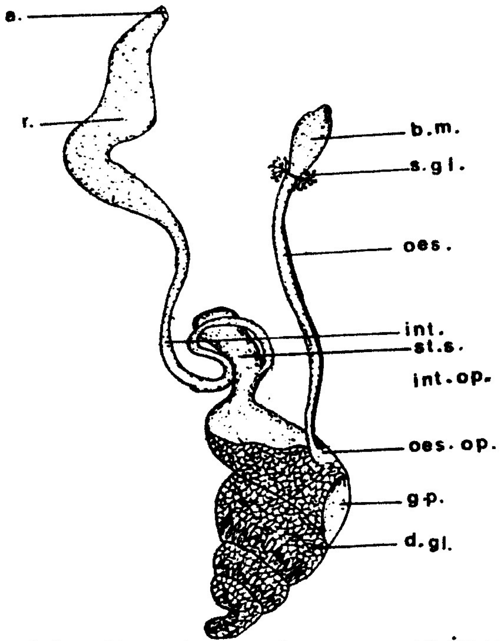


Fig. 1: Drawing of the digestive system as seen in a dorsal view. 40 X

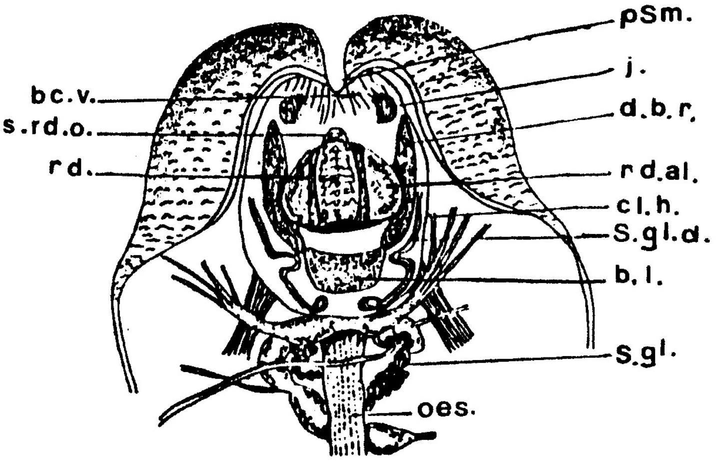


Fig. 2: The buccal mass opened longitudinally, from the middorsal side with the two flaps reflected on both sides. 140X

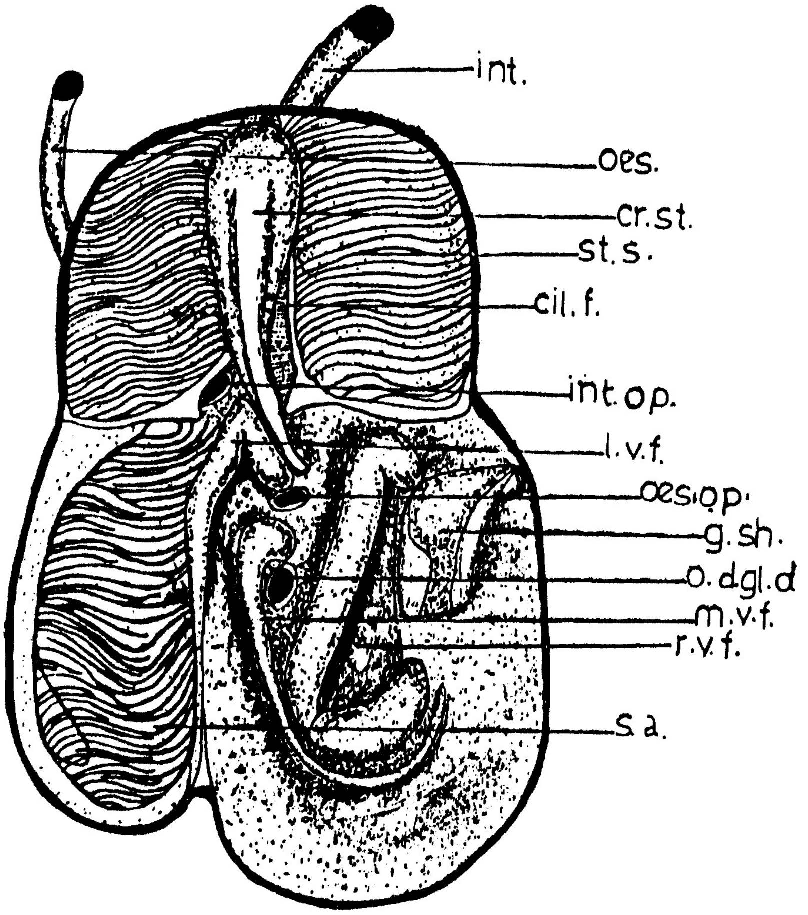


Fig. 3: Drawing of the stomach, after making a longitudinal dorsolateral incision, and reflecting the flaps. 400X

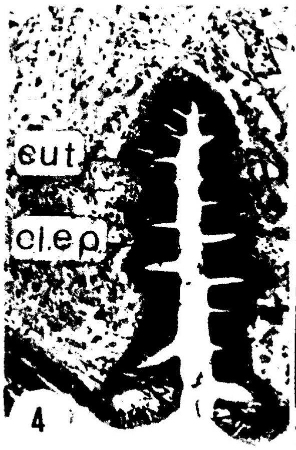
Fig. 4: Photomicrograph of T. S. through the peristome. 150X

Fig. 5: Photomicrograph of a T. S. through the jaw. 3500X

Fig. 6: Photomicrograph of a T. S. of the buccal mass through the odontophoral region showing buccal ridge. 200X

Fig. 7: Photomicrograph of a L. S. through the radular sac, showing sub-and superaradular epithelia. 200X

Fig. 8: Photomicrograph of a T. S. through the pro-oesophagus showing the goblet cells. 700X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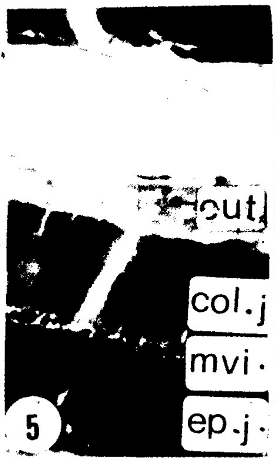
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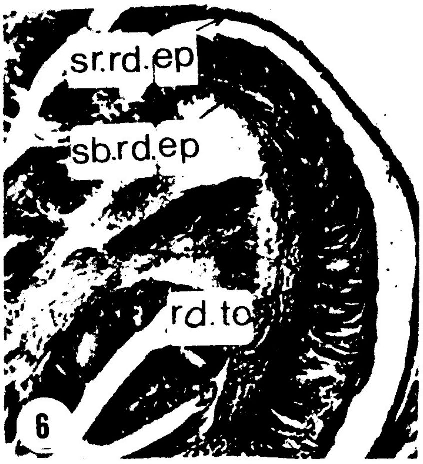
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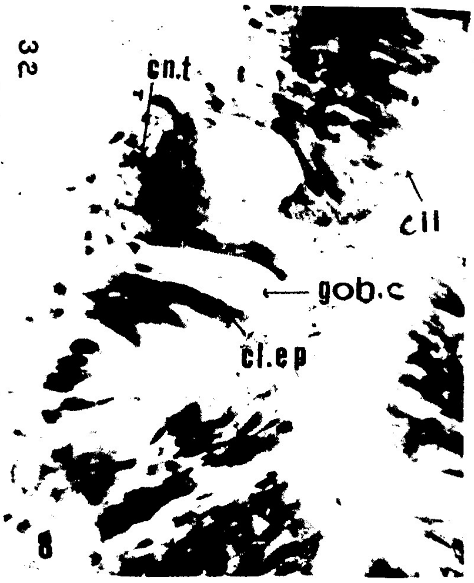
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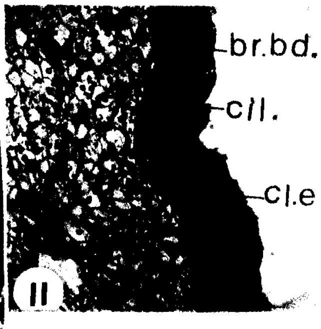

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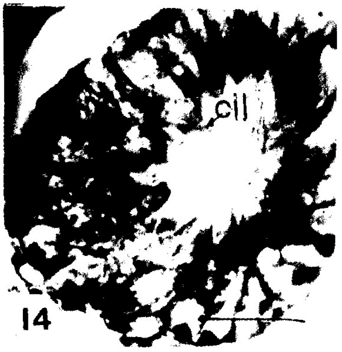

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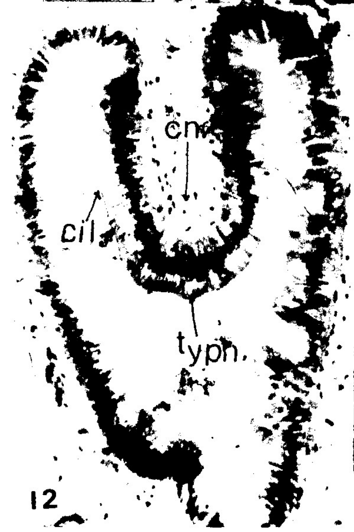

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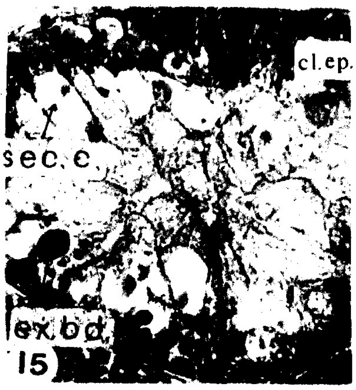

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Fig. 9: Photomicrograph of a T. S. of stomach, through the gastric portion, showing the digestive gland, digestive gland ducts, hepatic vestibule & sorting area. 80X

Fig. 10: Enlarged portion of the wall of the gastric portion showing the secretory cells. 1400X

Fig. 11: Photomicrograph of a T. S. through the wall of the style sac, showing the brush border-like cilia. 200X

Fig. 12: 'Photomicrograph of a T. S. passing through the prointestine showing the typhlosole. 200X

Fig. 13: Photomicrograph of an enlarged portion of the same as in fig. 12. 3500X

Fig. 14: Photomicrograph of a Section through the tubules of the salivary gland. 3500X

Fig. 15: Photomicrograph of a section through the digestive gland. 1300X

LIST OF ABBREVIATIONS

a	anus	int. op.	intestinal opening
b. ca	buccal cartilage	j.	jaw
b. l	buccal ledge	l. m. l	longitudinal muscle layer
br. bd	brush border	l. v. f.	left ventral fold
b.m	buccal mass	m. v. f.	middle ventral fold
b. v	buccal vestibule	mo.	mouth
c. m. l	circular muscle layer	mvi.	microvilli
cil.	cilia	o. b. r.	outer buccal ridge
cil.g	ciliary groove	o. d. gl. d.	opening of digestive gland duct
cil. f	ciliary furrow	o. s. gl. d.	opening of salivary gland duct
cl. ep.	columnar epithelium	o. r. al.	outer layer of alary process
cn. t.	connective tissue	oes.	oesophagus
col.j.	inner layer of cuticular columns of jaw	oes. op.	oesophageal opening
cr.st	crystalline style	r.	rectum
cut.	cuticle	r. v. f. rd.	right ventral fold
cut. j	outer cuticular layer of jaw		radula
d. b. r.	dorsal buccal ridge	rd. al.	alary process of radula
d. gl.	digestive gland	rd. col.	radular collostyle
d. gl. d.	digestive gland duct	rd. te.	radula teeth.
d. gl. t.	digestive gland tubule	s. a.	sorting area
ep. j.	chitinogenic columnars epithelium of jaw	gl.	salivary gland
ex. bd.	excretory bodies	s. gl. d.	salivary gland duct
g. p.	gastric portion	sb. rd. ep.	subradular epithelium
g. s.	gastric shield	sec. c.	secretory cell
gob. c	goblet cell	sr. rd. ep.	supraradular epithelium
h. v.	hepatic vestibule	srd. o.	sub radular organ
i. b. r.	inner buccal ridge	st. s.	style sac
i. rd. al.	inner layer of alary process	t. p.	tunica propria
int.	intestine	typh.	typhlosle

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دراسات مورفولوجية على قوقع المياه العذبة من جنس *كليوباترا بليمويدس* (أماميات الخياشيم) ١- الجهاز الهضمى

مجدى توفيق خليل ـ رضا حسن على
قسم علم الحيوان - كلية العلوم - جامعة عين شمس

تم لأول مرة دراسة التشريح البين والدقيق للجهاز الهضمى لقوقع "كليوباترا بليمويدس" باستفاضة، كما نوقشت النتائج مع ما سبق من دراسات مماثلة على قواقع أخرى لنفس المجموعة. على الرغم من أن الجهاز الهضمى لقوقع "كليوباترا بليمويدس" يتفق بصفة عامه مع التركيب الأساسى لمثيله فى القواقع الأخرى من نفس المجموعة إلا أن الدراسة قد اهتمت بصفة خاصة بالتركيز على ما قد يوجد من اختلافات فى النوع أو الجنس بالنسبة لتركيب الكتلة الفميه والمريء والمعدة والأمعاء والمستقيم والغدد اللعابية والهضمية.