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Structural and Ultrastructural investigation on the digestive tubules of the freshwater mussel *Caelatura parreyssi* (Family: Unionidae) (Philippi, 1848) from the River Nile (Egypt).

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ABSTRACT

The current investigation is concerning with the studying of the histology and ultrastructure of the digestive gland of the freshwater bivalve *Caelatura parreyssi* using light and transmission electron microscopes. This study was carried out on ten healthy clams, which were collected from the River Nile in Egypt. The specimens were dissected and small pieces of the digestive gland were selected out and processed for histological and ultrastructural examinations. The histological results revealed that the digestive gland of *C. parreyssi* is composed of numerous tubules, which are separated by connective tissues. Three types of cells were identified lined the digestive tubules; the digestive, excretory and crypt cells. Concerning the ultrastructural examination, digestive vesicles of different stages were seen that represent the characteristic features of the digestive cells. Excretory cells were distinguished by their excretory granules, whereas crypt cells showed spherules of calcium, which areconsidered also as characteristic ultrastructural feature.

In conclusion, the present study revealed that the digestive gland of the freshwater bivalve *Cealatura parreyssi* has many functions covering the digestion, secretion and excretion processes. Moreover, it has a detoxification role.

INTRODUCTION

Freshwater bivalves had received special attention in the last three decades (Mandahl-Barth, 1988; Graf, 1998; Ibrahim *et al*., 1999; Bogan, 2004; Scholz and Glaubrecht, 2004). Freshwater bivalves belong to family Unionoidae is conspicuous member of lentic and riverine habitats worldwide. The main aspects of the general biology of the Unionoidae were recently reviewed by Aldridge (1999), Graf and Foighil (2000), Araujo *et al.* (2005), Garo (2006), Graf and Cummings (2006a). Unionidae species are found often in dense aggregations and filter out large quantities of blue-green algae, bacteria and fine particulate organic molecules. Thus, Unionoid mussels play a major part in the functioning of many freshwater ecosystems. Moreover, Unionoids account for more than 90% of the energy content of the bottom fauna in the River Nile (Bogan, 2004; Graf and Cummings, 2007).

The anatomy and histology of the alimentary tract of freshwater Unionoida had been the subject of many investigations (Fawaz, 2004; Tantiwisawaruji *et al*., 2011). Studies on the functional morphology of feeding and digestion in the Bivalvia had contributed useful information about the class. The use of digestive gland as an indicator of phylogentic relationship led Purchon (1978 and 1987) to propose two new subclasses and five new orders. Moreover, Morton (1983) reported that the use of digestive tract morphology to discern taxonomic relations in the Bivalvia was useful.

The digestive gland had been mainly described in various species of marine bivalves (Morton, 1984; Aboul-Dahab *et al*., 1994; Winstead, 1998 and Ali, 2006). On the other hand, limited studies of the digestive gland had been conducted on freshwater bivalves. Moreover, the histological description of the digestive tubules had been illustrated only at the light microscopical level (Awad, 1995; Fawaz, 2004; Gabri*et al.,* 2005; Bhosale, 2017). Meanwhile, the fine structure of the digestive tubules of bivalves had been examined only in some marine species, such as *Cardium edule* (Owen, 1970); *Arctica islandica* (Palmer, 1979); *Mercenaria mercenaria* (Robinson and Langton, 1980) and *Crassostrea virginica* (John, 1995).

Little is known about the ultrastructure of the digestive gland of freshwater bivalves (Sumner, 1966; Pal, 1971 and 1972). In the same context, no previous ultrastructural studies had been documented on the freshwater bivalves or Unionoidae live in Egypt. Therefore, the aim of the present study is to investigate the digestive gland of *Caelatura parreyssi* using light and transmission electron microscopy techniques to identify the different cells in the digestive tubules and to provide preliminary information on the fine structure of these cells with relation to their functions.

MATERIALS AND METHODS

Specimen collection:

Ten healthy active specimens of the freshwater bivalve *Caelatura parreyssi* were collected from Ayatt canal, Giza governorate. They were preserved in a container with some water from the canal, and then transported to the laboratory at the Department of Biological and Geological sciences, Faculty of Education, Ain Shams University.

Histological preparation:

For light microscopy, the valves were opened and their digestive glands were rapidly dissected out and fixed in aqueous Bouin's solution for 24 hours. After that, the fixed tissues were dehydrated in a grade series of ethonal, and then cleared in terpineol for at least 48 h. Finally, the tissues were embedded in paraffin wax (Howard and Smith, 1983). Sections of 5-6 μm-thick were cut and stained with Ehrlich's acid alum hematoxylin and counter stained with eosin (Bancroft and Gamble, 2002). Slides were examined with light microscope (Olympus Bx 41, Tokeo, Japan) and photomicrographs were made as required.

Electron microscopic preparation:

For transmission electron microscopic preparation, very small pieces of the digestive gland were immediately fixed in cold mixture of 4% formalin and 1% gluteraldhyde and then washed in phosphate buffer (pH7.3) for 24 h. The fixed tissues were transferred into phosphate buffer, two changes for one hour, and post-fixed in 1% Osmium tetroxide for 24 hours and then washed in the same buffer. The specimens were dehydrated in ascending grades of ethanol, cleared in propylene

oxide and embedded in resin (Dykstra *et al*., 2002). Ultrathin sections (80 mm) were cut and mounted on copper grids. The sections were stained with uranyl acetate and lead citrate, examined and photographed with a JEOL-TEM-1400 EX-electron microscope at the Regional central for Mycology and Biotechnology (RCMB), AL-Azhar University.

RESULTS

Histological observations:

The greenish – brown digestive gland of *Caelatura parreyssi* occupied much of the antero-dorsal visceral mass. This compound tubular gland connected to the stomach by a duct system. It consisted of numerous blind-ending tubules which were separated by a connective tissue that infiltred by muscle fibres and haemocytes. The digestive tubules were either round or oval in cross-section (Fig. 1). The wall of each tubule appeared lined with three main cell types; digestive, excretory and crypt cells, which were irregularly grouped around the lumen of the tubule (Fig.2). Digestive cells were more numerous with variable heights and shapes being tall columnar or irregular cuboid in shape. Their nuclei were oval and elongated in shape and were basally located. Their cytoplasm appeared acidophilic and contained numerous vesicles of different sizes (Figs. 2 & 3). The apical portion of this cell is facing the lumen contained numerous microvilli (Fig.2).

Excretory cells were present in between the digestive cells; but being shorter and less numerous than the digestive cells. These cells were globular in shape and had basal nuclei. Their cytoplasm contained vacuoles of different sizes in a supra-nuclear position (Fig. 2).

Crypt cells were less numerous and pyramidal in shape with elongated basal nuclei. Their cytoplasms were more homogeneous and darker than the neighbouring digestive cells. These cells formed crypt which were localized in the tubule corner (Figs. 2 & 3).

Electron microscopic observations:

Digestive cells: Transmission electron microscopy examination showed that, the digestive cells were tall columnar cells bearing microvilli on their free surfaces. Pits could be seen at the base of microvilli (Fig. 4). In addition, the lateral plasma membranes of these cells showed prominent desmosomes. Their nuclei were basal, elongated or oval in shape, and possessed rounded nucleoli, electron dense heterochromatin, finally euchromatin and wavy double layered nuclear membranes (Figs. 4 & 5).

The cytoplasm of these cells revealed the presence of two active complexes, which were localized in supranuclear region of the cell and arranged more or less concentrically to enclose an extensive cup-shaped Golgi region. This cup-shaped structure appeared formed of several coils (Figs. 5 & 6). The mitochondria were abundant in the apical cytoplasm of these cells as well as around the basal nuclei, which being spherical or ovoid in shape with highly organized tubular cristae (Figs. 4 & 5).

The rough endoplasmic reticulum appeared extended throughout the basal and middle regions of the digestive cells with their cisternae run mainly parallel to each other (Figs. 4, 5 & 6). In addition, several membrane vesicles of various sizes and vacuoles were also observed in the cytoplasm of the digestive cells (Figs. 4 & 5). These vesicles contained highly electron dense materials, which often occupy the entire vesicular spaces. There were different types of vesicles found in the cytoplasm

but it was difficult to classify them according to their content. It could be suggested that these vesicles represented different functional stages (Figs. 5 & 6).

Excretory cells: As illustrated in micrographs (Figs. 7, 8 & 9), the cytoplasm of these cells was rich in globular excretory granules of various sizes appeared mainly at their apical surfaces. These granules appeared single or connected and joined with each other. Moreover, these cells had basal nuclei, which surrounded by distinct irregular nuclear envelopes and possessed nucleoli and chromatin materials (Figs 7 & 8). Short microvilli extending from their apical surfaces were clearly seen (Figs. 8 & 9). Mitochondria were numerous in the apical cytoplasm (Fig. 7). The rough endoplasmic reticulum was also appeared in the cytoplasm of these cells (Figs. 7 & 8). Electron dense excretion and wide spaces between them and membranes could be seen in Figure (9).

Cryptcells (basophilic cells): these cells were pyramidal in shape and inter-connected apically by layered basal lamina. It had a broad base containing a large chromatin-rich nucleus and is filled with rough and smooth endoplasmic reticulae, mitochondriae, calcium spherules and ribosomes (Figs. 10, 11 & 12). The rough endoplasmic reticulum appeared surrounding areas of the cytoplasm. Also, it was associated with granular vacuoles and calcium spherules. Free ribosomes, mitochondria and Golgi apparatus were present between calcium spherules of different sizes. The calcium spherules of the early developmental stage showed a central granule and granulofibrillar materials that were randomly dispersed in a large matrix. Small spherules composed entirely of electron dense materials had also been observed. These were considered immature spherules (Fig. 10). The Golgi apparatus were composed of 2-3 dictyosomes in synchronous secretory activity. The cytoplasm of these cells contained numerous free ribosomes (Figs. 10 & 11).

DISCUSSION

Freshwater bivalves represent very important items to the bottom-feeder fishes in the ecosystem and an important trophic level for environmental stability. Freshwater mussels are also of special interest due to their imperiled status (Lydeard *et al*., 2004; Strayer *et al*., 2004).

The main aspects of the general biology of freshwater bivalves had received a special attention in the recent years among the Egyptian Zoologists (Fawaz, 2004; Gabri *et al*., 2005). On the other hand, few previous ultrastructural studies had been documented on the freshwater bivalves or unionoids found in Egypt. Therefore, the current study was designed to examine the histology and ultrastructure of the digestive gland in freshwater mussel *Caelatura parreyssi* to clarify its characteristic cells and their functions. The present histological study showed that the digestive gland of *C. parreyssi* consists of a great number of digestive tubules, which connected with each other by connective tissues. Each tubule has a wide irregular lumen. The structure of the digestive gland and its ducts in *C. parreyssi* is basically similar to that of many other freshwater bivalves that described by Fawaz (2004), Gabri *et al*. (2005), Tantiwisawaruji *et al*. (2011), Bhosale (2017) and Vasanthi *et al*. (2017).

The current investigation showed that, three types of cells were identified in the digestive gland epithelium; the digestive, excretory and crypt cells. This result run parallel with those obtained by Winstead (1998) who identified three cell types; digestive, flagellated basophil and non flagellated basophil cells in the digestive tubules of *Crassostrea virginica*. Also, Frenzel (1893) recorded three kinds of cells in

the digestive diverticula in intertidal and subtidal oysters, *Crassostrea virginica* namely; granule, ferment and lime cells.

However, on the other filter feeding bivalves such as *Lyonsia hyaline* (Thomas, 1993), *Corbicula fluminea* (Awad, 1995), *Caelatura teretiuscula* (Fawaz, 2004; Gabri, *et al*., 2005), *Hyriopsis bilatus* (Tantiwisawaruji *et al*., 2011) and *Corbicula striatella* (Bhosale, 2017), the walls of their digestive tubules are lined by two types of cells; the digestive and basophilic cells (crypt cells). On the other hand, there is only one cell type in the tubules of some marine lambellibranchs such as *Arctica islandica* which has one cell type through a number of distinct phases in its life history as reported previously by Palmer (1979).

Thus, no unanimity on the number of cell typesexist in the digestive gland of freshwater bivalves.

The digestive cells in *C. parreyssi* were numerous and had variable shapes and heights. This result is in harmony with those obtained by Tantiwisawaruji *et al*. (2011) in the freshwater bivalve *Hyriopsis bilatus*. The author suggested that the height of the digestive cells increased during the apocrine secretion, then decreased after the elimination of spherules in the lumen. Moreover, the digestive cells of the digestive tubules ingest particles by endocytosis and digest them intra-cellularly (Owen, 1955, 1970, 1973 and Pal, 1972). In this process, nutrients are absorbed in the basal area and passed into the haemocoel, whereas waste products are aggregated at the apex of the cell, released as waste spherules into the lumen of the tubule and transported by ciliary currents out of the digestive diverticula into stomach (Thomas, 1993).

The present study has shown that, the excretory cells are present in between the digestive cells but being shorter and less numerous than the digestive cells. These cells possessed more than one large vacuole in the supranuclear position. These results concide with those observed by Taieb and Vicente (1999) in *Aplysia punctata* and De Vico, *et al*. (2017) in *Theba pisana*.

In the current work, the third cell type; the basophilic triangular-shaped crypt cells had a single basophilic nucleus. This is in accordance with many species of bivalves, which contain crypt cells separating digestive cells within the digestive tubules, such as *Lyonsia hyaline* (Thomas, 1993) and *Hyriopsis bilatus* (Tantiwisawaruji *et al.,* 2011). Palmer (1979) decided that the basophilic cells may serve in digestive cell replacement; this is due to the presence of mitotic figures. Whereas Simkiss and Mason (1984) reported that the crypt cells possessed a special function in detoxification of pollutants.

Concerning the ultrastructure of digestive tubules of *C. parreyssi*, the current result showed that the digestive cells have microvilli on their free surfaces and pits at the bases of the microvilli. The ultrastructural details of cell organelles of the digestive cells were mostly similar to that of the digestive cells of other marine bivalves such as *Cardium edula* and *Crassostrea virginica* that examined by Owen (1970), John (1995) and Winstead (1998), as well as number of gastropods like, *Acnatina fulica* (Chaki and Misra, 2004), *Adelomelon beckii* (Arrighetti *et al*., 2015), *Zidona dufresnei* (Ojeda *et al*., 2015), *Laevicau lisalta* (Nath*et al*., 2015) and *Theba pisana* (De Vico *et al*., 2017). The presence of membrane invagination at the apical surface of the digestive cells suggests a process of pinocytosis of food particles as reported previously by Taieb (2001). The present observations revealed the extensive elaboration of the Golgi bodies that occurred in the digestive cells. These results run parallel with those recorded in *Achatina fulica* (Chaki and Misra, 2004) and *Laevicaulis alta* (Nath*et al*., 2015). Moreover, mitochondria were abundant around

the basal nucleus as well as the apical portion of the digestive cells of *C. parreyssi*. This result concides with Walker (1970), Boghen and Farley (1974), Dimitriadis and Liosi (1992) and Chakiand Misra (2004). The number and position of mitochondria generally indicates the activity of the cell concerned. Nath *et al*. (2015) suggested that the elaboration of mitochondria towards the apical portion of the cell point to the active state of the digestive cells. Concentration and distribution of endoplasmic reticulum were homogenous around the nucleus of the digestive cells of *C. parreyssi*. The most characteristic feature of the digestive cells of *C. parreyssi* was the presence of numerous membrane-bound vacuoles. This is also in accordance with the result obtained in other molluscs by Wilson and La Touch (1978), Robinson and Langton (1980), Yousef and El kassas (2013) and Nath *et al*. (2015). Nath *et al*. (2015) suggested that the numerous coated vacuoles in the cell point towards the active state of the cell.

Excretory cells were clearly distinguished by the presence of excretory granules of various sizes at their apical surfaces. Fusion of small granules with one another occurs in the apical region of the cell. The ultrastructural details of excretory cells concide with the excretory cells of *Aplysia punctate*, which were examined by Taieb and Vicente (1999).

The present study showed that the crypt cells of *C. parreyssi* are characterized by pyramidal shape and possessed a conspicuous nucleus, well developed rough endoplasmic reticulum and extensive Golgi complex. These observations run parallel with those obtained by Sumner (1966), Owen (1973) and Winstead (1998) in other bivalves. In addition, crypt cells were clearly characterized with the presence of calcium spherules. This is in accordance with Almedros and Porcel (1992), Luchtel *et al*. (1997) and Nath *et al*. (2015) where they considered calcium spherules to be characteristics of crypt cells. Role of calcium spherules is speculated to be associated with various physiological processes such as regulation of the luminal pH (Burton *et al.*, 1987), regenerative processes (Luchtel *et al*., 1997). It is evident that the nucleus of the calcium spherules originates from the Golgi vesicles as well as from the Golgi sacculus surrounding areas of cytoplasm. The relationship between Golgi complex, rough endoplasmic reticulum, ribosomes, mitochondria and calcium spherules indicated that all these organelles are involved in the formation of the calcium spherules. Thomas (1993) found that the crypt cells of marine bivalve *Lyonsia hyalina* had various shapes. Morton (1983) explained these phenomena as these cells undergo structural changing during the digestive cycle. Crypt cells are sometimes secretory and may serve as digestive cell replacements or may perform both functions (Owen, 1970, 1973; Pal, 1972 and Morton, 1983).

In conclusion, the present findings of the histology and ultrastructure of the digestive gland cells of *C. parreyssi* are in conformity to that of other bivalves and gastropods. During the digestive cycle, digestive and crypt cells morphologically changes in relation to ion metabolism and intercellular digestion, respectively. Finally, the digestive gland of *C. parreyssi* appears as a major absorptive and secretory organ, which may also possesses excretory and detoxifying functions.

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EXPLANATION OF FIGURES

Figures 1-3: Photomicrographs of transverse sections of the digestive gland of *C. parreyssi* stained with Hx& E.

Fig. (1): Illustrating the digestive tubule which lined with epithelial cells (E) arranged around a narrow irregular lumen (L).

Figs (2 & 3): Enlarged portion of the above figure showing different cells of the digestive tubules; digestive cells (DC), excretory cells (EC) and crypt cells (CC) with brush border (BB) on its free surface. The basement membrane (BM) and lumen (L) are clearly seen.

Figures 4-12: Transmission electron micrographs of the digestive gland of *C. parreyssi*.

Fig. (4): Showing the digestive cell with microvilli (Mv) on its free surface projecting towards the lumen (L) with pits (P) between them, gap junction (GJ) are seen. Various sized vacuoles (V) and rough endoplasmic reticulum (RER) scattered in the cytoplasm. The nucleus (N) with irregular nuclear membrane (NM) are also observed.

Fig. (5): High magnified portion of the digestive cell illustrating an irregular nucleus (N) having around nucleolus (Nu), weavy nucler membrane (NM), heterochromatin (Hc) and euchromatin (Ec). Rough (RER) and smooth (SER) endoplasmic reticula, mitochondria (M), Golgi complex (GC), gap junction (GJ) and vacuoles (V) are also noticed.

Fig. (6): Illustrating magnified part of the digestive cell representing rough endoplasmic reticulum (RER) in the form of elongated and parallel cisternae studded with ribosomes, smooth endoplasmic reticulum (SER) scattered into the cytoplasm, mitochondria (M) in the form of rounded or ovoid configuration, Golgi complex (GC) composed of parallel curved cisternae, different vacuoles (V) and a part of the nucleus (N) with its distinct nuclear membrane, are also noticed.

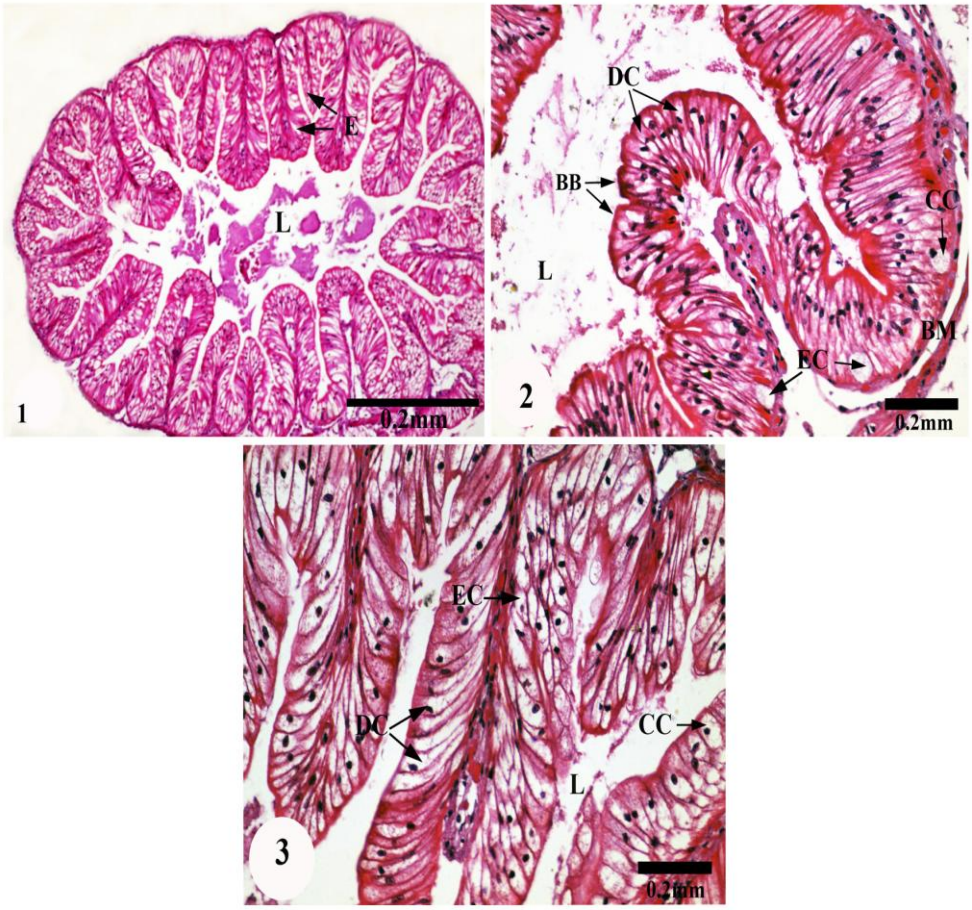
Fig. (7): Magnified part of the excretory cell illustrating rounded and ovoid mitochondria (M) being numerous in the apical cytoplasm near the irregular nucleus (N), with distinct mitochondrial membranes, mitochondrial ridges and matrix. Besides, extensive elaboration of rough endoplasmic reticulum (RER) in the form of parallel cisternae localized nearthe nuclear membrane (NM). Globular scattered excretory granules (EG) are also seen.

Fig. (8): Showing excretory cell which has numerous excretory granules (EG) of various sizes at the apical surface of the cell,they appeared singly or joined together,apical microvilli (MV) and pits (P) are also illustrated. The nucleus (N) with distinct rounded nucleolus (Nu) and irregular nuclear membrane (NM). Notice the rough endoplasmic reticulum (RER) scattered in the cytoplasm.

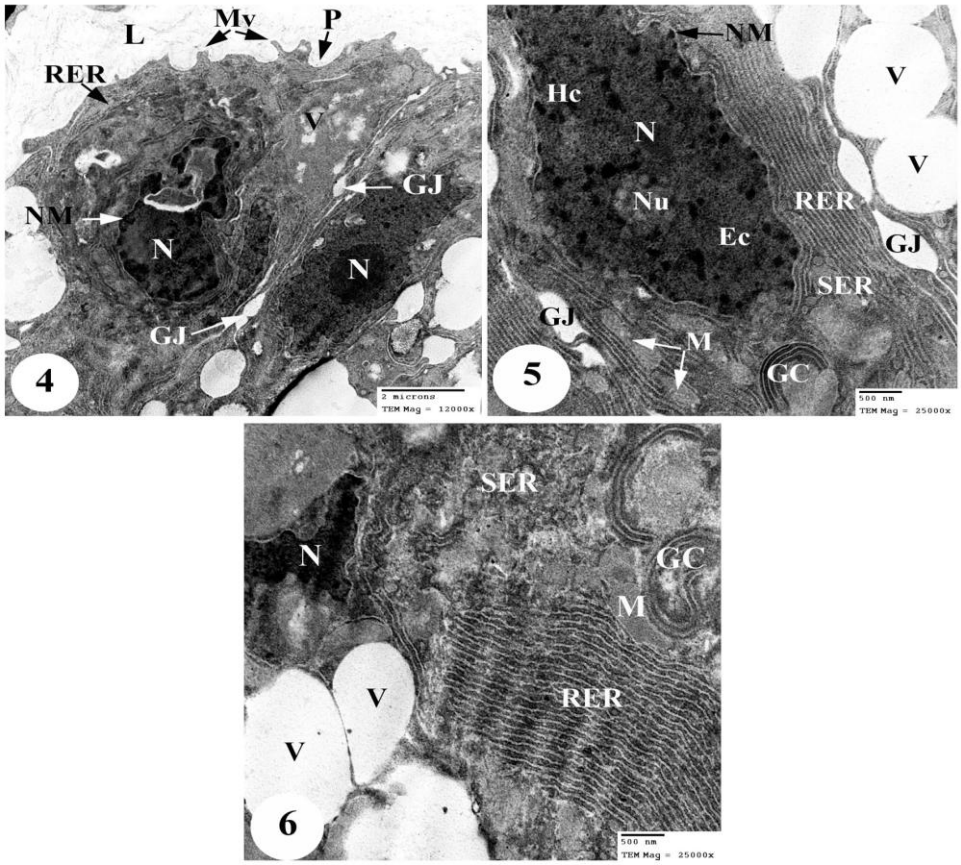
Fig. (9): Illustrating the presence of microvilli (Mv) at the tip of both the digestive and excretory cells. Variable sized of excretory granules (EG) scattered in the cytoplasm at the microvilli bases of excretory cell, whereas small vesicles (V) were seen at microvilli bases of the digestive cell.

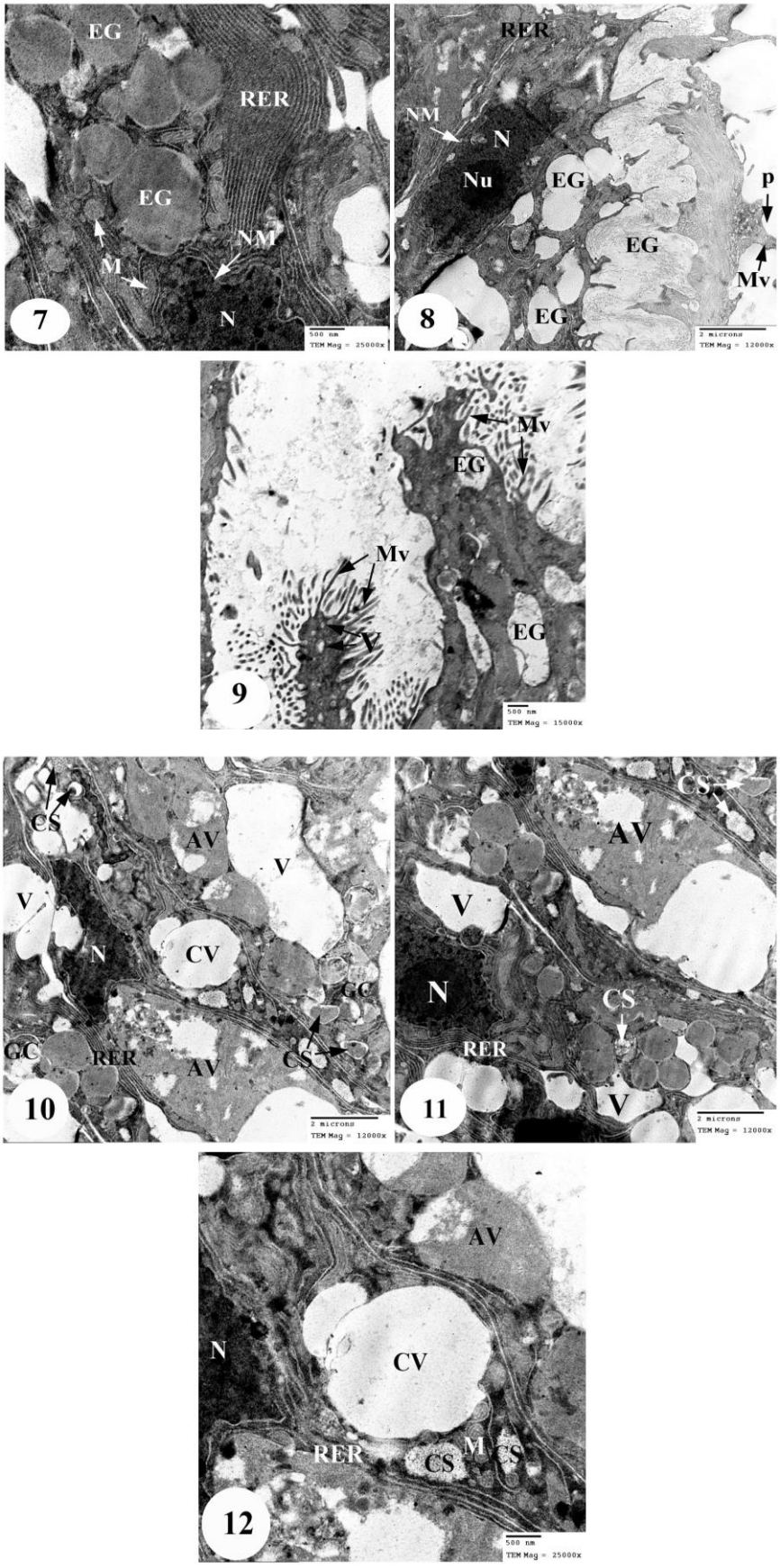
Fig (10): Showing the characteristics cytoplasm and nucleus of the crypt cell. It has a broad base containing chromatin rich nucleus (N). Rough endoplasmic reticulum (RER), vacuoles (V) and Golgi complex (GC) scattered in the cytoplasm. Small calcium spherules (CS) contain electron dense material, autophagic vacuole (AV) and large central vacuole (CV) are also seen.

Figs. (11& 12): Magnified part of the above figure revealing different sizes of calcium spherules (CS). Numerous mitochondria (M), rough endoplasmic reticulum (RER), large central vacuole (CV), autophagic vacuole (AV) and irregular nucleus (N) are also illustrated.



Figs. 1-3: Photomicrographs of transverse sections of the digestive gland of *C. parreyssi* stained with Hx& E.





Figs. 4-12: Transmission electron micrographs of the digestive gland of *C. parreyssi*.

ARABIC SUMMARY

دراسات هستولوجية وتركيبية دقيقة للغدة الهضمية فى محار الماء العذب (Family: Unionidae) *Caelatura parreyssi* من نهر النيل فى مصر

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تهدف الدراسة الحالية لوصف التركيب الهستولوجى والتركيب الدقيق للغدة الهضمية فى محار الماء العذب *Caelatura parreyssi*. أجريت هذه الدراسة على عشرة محاريات تم جمعها من نهر النيل فى مصر ثم تشريحها للحصول على الغدة الهضمية. تم فحصها بالميكروسكوب الضوئى والميكروسكوب الإلكترونى وأظهرت النتائج أن الغدة الهضمية تتكون من عدد كبير من الأنيبيبات الهضمية التى ترتبط معاً بنسيج ضام. كما تم تحديد ثلاثة أنواع من الخلايا فى طلائية هذه الأنيبيبات وهى الخلايا الهضمية والخلايا الإخراجية والخلايا الهرمية.

وأشتملت الدراسة الحالية على وصف التركيب الدقيق لهذه الخلايا حيث أظهرت أن وجود عدد كبير من الحويصلات الهضمية هى من السمات المميزة للخلايا الهضمية، كما أن الخلايا الإخراجية تحتوى على حبيبات أخراجية بينما الخلايا الهرمية تتميز بوجود كرات الكالسيوم.

وبذلك أستدلت الدراسة النسيجية على تعدد وظائف الغدة الهضمية حيث لها دور فى عملية الهضم والإخراج علاوة على دورها فى التخلص من السموم.