Histological Structure of *Strombus gigas* (Gastropoda: Caenogastropoda: Strombidae)

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ABSTRACT

A histological study of *Strombus gigas* was analyzed with samples of the proboscis, eye antenna, foot, penis, anus, ctenidium, style sac, and kidney. We observed five types of epithelial tissue: Simple columnar epithelium (foot, anus, ctenidium, kidney); pseudo-stratified epithelium (proboscis, eye antenna); glandular epithelium (foot, kidney), simple cuboidal epithelium (style sac); and transitional epithelium (style sac and anus). The simple cuboidal and the simple columnar epithelia are ciliated. Two types of connective tissue were observed: fibrous and loose. The fibrous connective tissue was found in all structures analyzed in this study, except in the style sac, which showed loose connective tissue and on glandular tissue in the kidney.

KEY WORDS: Epithelial tissue, histology, tissue, Queen Conch, *Strombus gigas*.

Estructura Histológica de *Strombus gigas* (Gastropoda: Caenogastropoda: Strombidae)

Se realizó un estudio histológico de *Strombus gigas* con muestras de probóscide, antena del ojo, pie, pene, ano, ctenidio, saco del estilete cristalino y riñón. Se observaron cinco tipos de tejido epitelial: Epitelio columnar simple (pie, ano, ctenidio, y riñón); epitelio pseudo-estratificado (probóscide y antena del ojo); epitelio glandular (pie y riñón); epitelio cúbico simple (saco del estilete cristalino); y epitelio de transición (saco del estilete cristalino y ano). Los epitelios cúbico simple y columnar simple presentan cilios. Dos tipos de tejido conectivo se observaron: fibroso y laxo. El tejido conectivo fibroso se localizó en todas las estructuras analizadas en este estudio, excepto en el saco del estilete cristalino, que muestra tejido conectivo laxo y en el tejido glandular en el riñón.

PALABRAS CLAVES: Epitelio, histológico, caracol, *Strombus gigas*.

INTRODUCTION

The Queen conch *Strombus gigas* Linnaeus, 1758 (Gastropoda: Caenogastropoda: Strombidae) is a marine resource of commercial importance to several Caribbean countries (Frenkiel and Aldana-Aranda 2003), second only to the spiny lobster. Its value comes from the trade of both meet and shell (Brownell and Stevely 1981). Appeldoorn (1994) reported for the period of 1988 to 1991 a catch of 4,000 metric tonnes (t) with a value of 40 million US Dollars. CITES (2003) informs that between 1993 and 1998, the total annual landings of Queen conch meat ranged between 6,520 t and 7,369 t. Since then, annual landings have fallen and were 5,554 t in 1999, 4,598 t in 2000, and 3,132 t in 2001.

S. gigas maintains a high pressure of fishing that has reduced its populations in all its distribution (Creswell 1994, Stoner and Davis 1997) to such degree that it has been included in Appendix II of CITES since November 1992 and has been classified since 1994 in the Red List of Threatened Animals of IUCN as Commercially Threatened (CITES 2003).

Since the 1960s, more than 600 scientific articles have been published related to *Strombus gigas* (Darcy 1981, Acosta 1994, Aldana-Aranda 2000, Ray-Culp 2002), even so, the information on its histology is limited. These studies are necessary to understand the physiology of these organisms for the acquisition of the food, absorption, locomotion, and to recognize tissue damage by parasites and contamination.

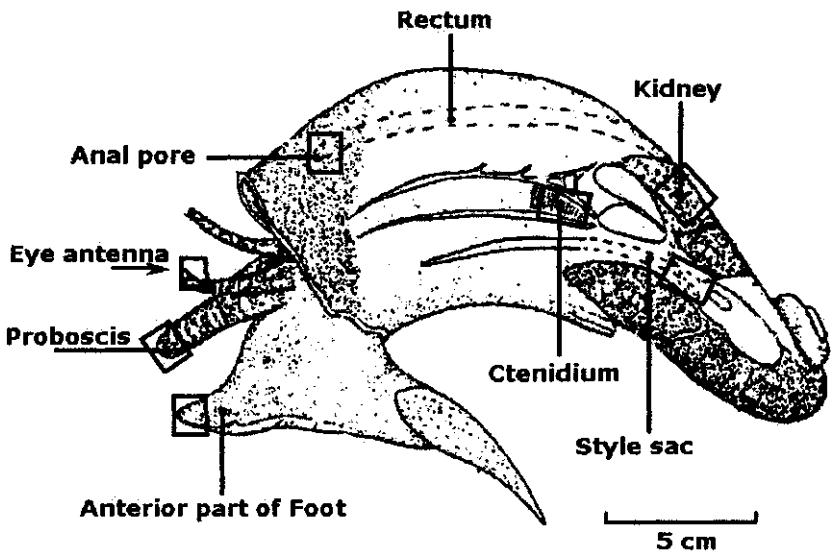
Some of this biological information is needed in mariculture operations (Berg Jr 1981). Aldana-Aranda et al. (2003) studied and compared the reproductive cycle of *S. gigas* and its gonadic development states from two Mexican regions. Avila-Poveda (2001) described tissue structures of some organs of *S. gigas*. Glazer and Quintero (1998) examined the viability and the capacity that has *S. gigas* of offshore region and nearshore region to undergo gametogenesis. Reed (1993, 1994, 1995a, 1995b) studied the reproductive anatomy and biology of the six Caribbean species of *Strombus* with an emphasis on androgynous males and masculinized females. Buckland (1989) inspected and evaluated during several months the amount of gametogenic tissue contained in the gonad of *S. gigas*. Egan (1985) examined to the microscopic tissue sections in cross section through the digestive gland and gonad of *S. gigas*, to give to scales of development and sexual maturity.

Actually, the producing countries of live aquatic animals required appropriate strategies that minimize potential health risks associated with seafood production, emphasizing the importance of general procedures for sampling and fixation and of gross and microscopic observations (Bondad-Reantaso et al. 2001).

In the case of *S. gigas,* that is presently overfished and in development of its aquaculture, it requires information applicable that will enable prompt and effective response to disease situations in its aquatic production. The objective of this study was to provide general histology aspects of the proboscis, eye antenna, foot, penis, anus, ctenidium, style sac, and kidney, focusing on the epithelia and connective tissue, with commentaries on its functions, as the basis of diagnostics futures, as well as to record-keeping.

MATERIALS AND METHODS

Three males and three females adults organisms of *S. gigas* (lip thickness, 14 - 16 mm), were collected on 22°22" North latitude and 89°41" West longitude at the Alacranes Reef, located to 135 km to the front of the coasts of the municipality of Progress, Yucatán, México. Each animal was removed from its shell and samples from 1 cm^3 were taken of proboscis, eye antenna, foot, penis, anus, ctenidium, style sac, and kidney (Figure 1). The samples were fixed in Davidson's fixative (Bondad-Reantaso et al. 2001) during four days. The samples were washed in 70 % ethanol, dehydrated in series of 70 %, 96 % and 100 % ethanol, and embedded in Paraplast® paraffin, a tissue embedding medium (m.p. 56 °C). Sections of 6 µm thick were cut and mounted on glass slides with Meyer's albumin. The stain was made with Harris's Hematoxylin - yellowish Eosin, regressive method (Howard and Smith 1983). All tissue slices was described at microscopic amplification of 400x and 1000x, and then was photographed using a high-resolution Sony CCD-IRIS color videocamera assembled to a light microscope Carl Zeiss MC-



6A.

Figure 1. General anatomy of *Strombus gigas*. In rectangle is located of dissection parts utilized in this study. The penis is not marked (modified of Little 1965).

RESULTS

Proboscis

The periphery of proboscis consisted of unciliated pseudostratified epithelial tissue, 60 - 80 µm in height. Their nuclei granulose are ovals 12 -

14 µm in length and 2 - 4 µm in wide, located in the central region of each cell. This epithelium has a basal lamina defined well. The connective tissue is fibrous dense (Figure 2).

Figure 2. Digital photography of the periphery of proboscis of *Strombus gigas*. Bl), basal lamina; Ft), fibrous connective tissue; Pce), pseudostratified columnar epithelium. Scale bar = 30 µm.

Eye antenna

A small tentacle protrudes from the end of the ocular peduncle (eyestalk). It is compound of fibrous dense connective tissue and the integument of this structure is unciliated pseudostratified epithelial tissue, 34 - 60 µm in height, with its granulose nuclei 9 - 12 µm in length and 2 - 3 µm wide. This epithelium displays a basal lamina defined by fibrous dense connective tissue (Figure 3).

Foot

It is constituted of dense connective tissue. Several arrangement of the integument was observed, in the foot side as in the foot sole. Integument of the foot side is ciliated glandular columnar cells (pedal glandule) with secretions between its folds. The epithelial cells measured 40 - 48 µm height, with cilia of 8 - 10 µm of tall and with granulose nuclei of 6 - 8 µm in length and 1 - 2 µm wide (Figure 4). The foot sole exhibits a ciliated columnar simple epithelium tissue. Each cell of this forward epithelium measured 20 - 36 µm in height, with cilia of 4 - 5 µm of tall and with granulose nuclei on basal position (Figure 5). The integument of the foot sole that is supported in the marine bed shows a pseudostratified epithelium tissue. This epithelium displays a lobule-twin arrangement, forming rounded crests and valleys. The cells of this epithelium are columnar 36 - 40 µm in height, with cilia 4 - 5 µm of tall and with granulose nuclei in basal position (Figure 6).

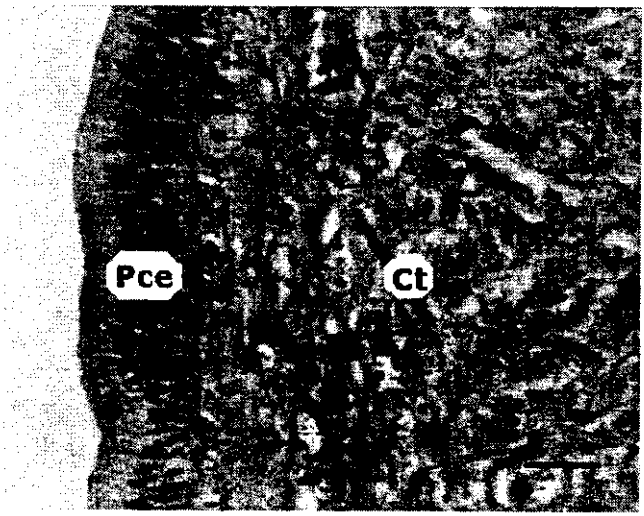


Figure 3. Digital photography of the integument of the eye antenna of *Strombus gigas*. Ct), connective tissue; Pce), pseudostratified columnar epithelium. Scale bar =40 µm.

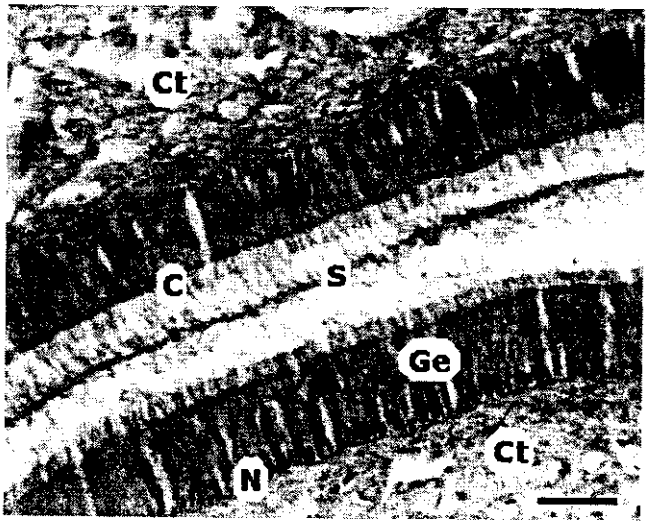


Figure 4. Digital photography of the integument folds on the gland pedal (foot side) in the anterior end of the foot of *Strombus gigas*. C), cilia; Ct), connective tissue; Ge), glandular columnar epithelium; N), basal nucleus of the columnar cell; S), secretions. Scale bar = 40 µm.

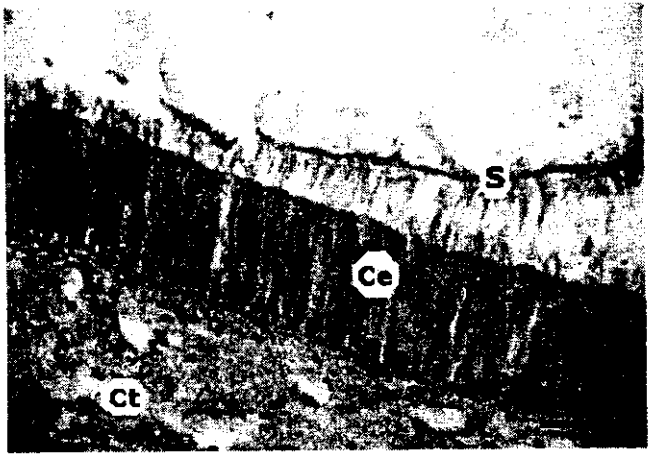


Figure 5. Digital photography of the epithelium lining the edge of the anterior end of the foot (foot sole contiguous to the foot side) of *Strombus gigas*. Ce), ciliated columnar epithelium; Ct), connective tissue; S), secretions. Scale bar = 30 µm.

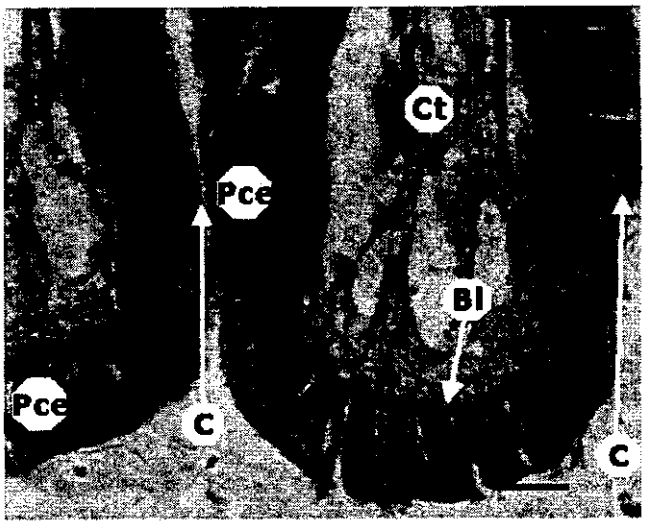


Figure 6. Digital photography of the epithelium lining the anterior end of the foot at the foot sole of *Strombus gigas*. Bl), basal lamina; C), cilia; Ct), connective tissue; Pce), pseudostratified columnar epithelium;. Scale bar = 40 µm.

Penis

Presented a ciliated glandular epithelium with sinuouse arrangement covered the spermatic groove. This epithelium measured 20 - 30 μm height, with cilia 4 - 6 μm of tall, and have abundant translucent secretory cells of a globular-cup shape (goblet cells) 12 - 20 μm in diameter. The connective tissue is dense type (Figure 7).

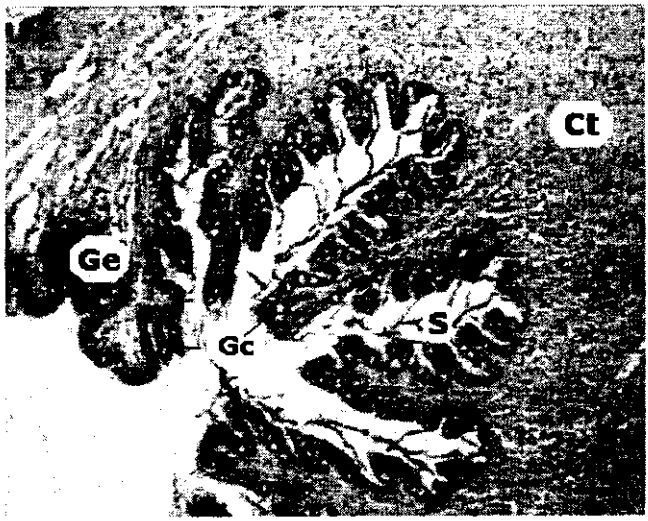


Figure 7. Digital photography of the spermatic groove of the penis of *Strombus gigas*. Ct), connective tissue; Gc), goblet cell; Ge), glandular epithelium; S), secretions. Scale bar = 60 μm.

Anus

Epithelial tissue covered internal and externally the anus. The external epithelium was a transitional type with undulations. Simple columnar ciliated cells composed the internal wall of the anus forming folds. These columnar cells measured 30-40 μm in height, with its cilia measured 16-40 μm of tall, and its nuclei on basal position measured 2-8 μm in diameter. The connective tissue is of the loose and dense type (Figure 8).

Ctenidium

Presented a series of tubular filaments supported by the mantle. The filaments were covered for ciliated columnar cells of 20-28 μm height. This epithelial cells have its granulose nuclei of 4-6 μm in diameter located in the middle region of each epithelial cell. The cilia were 30-40 μm of tall and was separated one of another one (Figure 9).

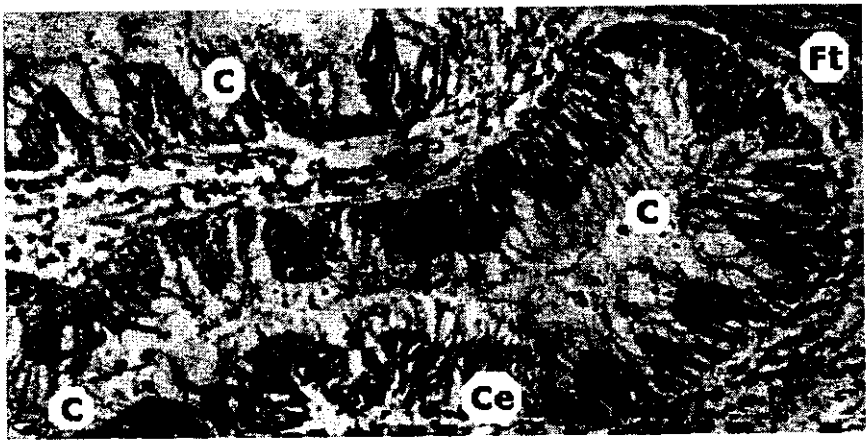


Figure 8. Digital photography of the folds internal of the anus of *Strombus gigas*. C), cilia; Ce), ciliated columnar epithelium; Ft), fibrous connective tissue. Scale bar = 80µm.

Style sac

The structure lodges the crystalline style. The style sac is formed by loose connective tissue and is covered by epithelium of transition. The gastric cavity this covered by epithelium cuboidal of 16 - 18 µm, with cilia of 18 - 22 µm of tall, very united and has the same size or more than the cuboidal cell. The cellular nucleus is round and granular of 6 µm located on middle region (Figure 10).

Kidney

It is a glandular tissue formed acini. This acinus was constituted of cells 30 µm of height with trapeze shape, with granular nucleus 7 µm in diameter on basal region. All glandular tissue is surrounded and closed by a simple columnar epithelial tissue of 20 - 40 µm of length, with its nuclei basal (Figure 11).

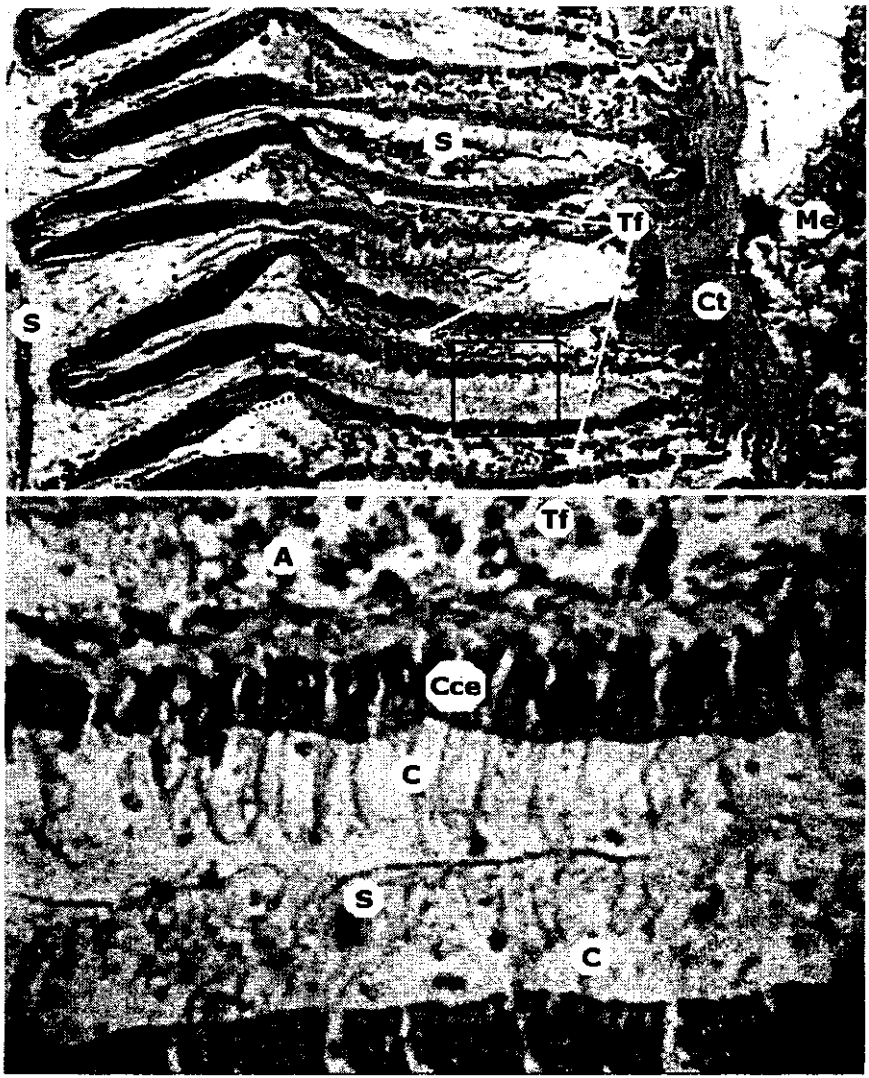


Figure 9. Digital photography of the ctenidium of *Strombus gigas*. A), amebocytes; C),cilia; Cce), ciliated columnar epithelium; Ct), connective tissue; Me), mantle; Tf), tubular filament; S), secretions. Scale bar = 180 µm and 30µm, respectively

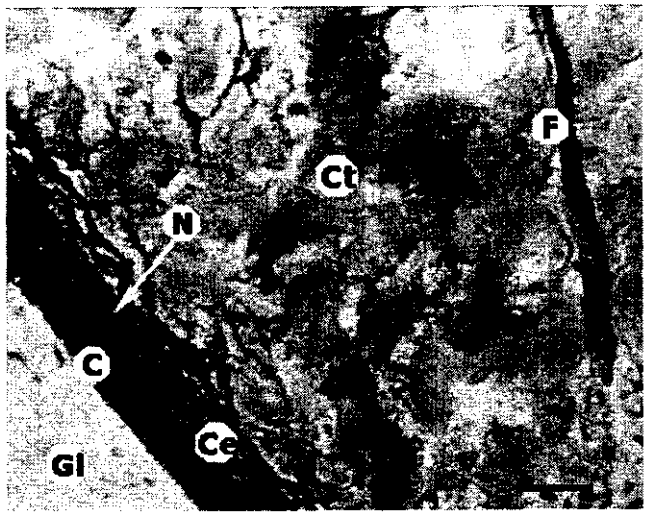


Figure 10. Digital photography of the gastric cavity at the style sac of *Strombus gigas*. C), cilia; Ce), gastric cuboidal epithelium; Ct), loose connective tissue; F), collagen fiber; Gl), gastric lumen; N), nucleus of the epithelial cell. Scale bar = 15 µm.

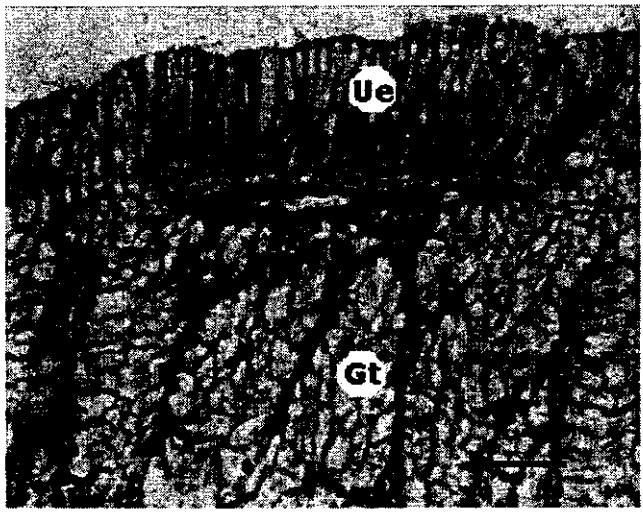


Figure 11. Digital photography of the kidney of *Strombus gigas*. Gt), glandular tissue; Ue), unciliated columnar epithelial tissue. Scale bar = 30 µm.

DISCUSSION

This study reports the general histology of *Strombus gigas*. The epithelial tissue is variable between the gastropods, given its diverse functions. Simkiss (1988) point out three types of cells: epidermal, ciliated and the glandular cells, with functions diverse according to the anatomical region of the animal, the species and its habitat. The epithelial tissue of the studied anatomical parts showed differences in the presence of cilia nuclei position and epithelium arrangement. These differences relate to its functions of protection, transcellular transports, secretion, absorption, lubrication, and distension of the epithelial tissue (Gartner and Hiatt 1997). Warren (1959) indicated that epidermis of the molluscs is generally of ciliate type, but that in this extensive phylogenetic group, these epidermis present variables, as they are glandular integuments and presence of named structures chromatophores. The epithelium of more predominance observed in this study was the columnar pseudostratified and the columnar simple (Table 1). The majority of epithelial tissue of the different studied parts is formed by the integument. The characteristic observed that the epithelium of the foot sole elaborate folds; according to Hughes (1986), it must be due to contractions of muscular waves that help to produce mucus through a temporary compression for the sliding on a substrate. The glandular epithelium that covered the spermatic groove of the penis is as stated by Reed (1995a, 1996b) a surface with goblet cells. The presence of great amount of goblet cells suggests the production of mucus to lubricate and to facilitate the movement of the sperm during the transference to the female (Hughes 1986). Reed (1995a, 1996b) indicated that this mucus could increase the adhesion of the surface of the spermatic groove to maintain itself during the copulation.

The presence of cilia in the epithelial tissue showed differences. The cilia of the ctenidium are longer than the same epithelial cell and are less numerous and more separated from one another; whereas the cilia of the columnar epithelia on the foot and on the anus, and the cilia of the cuboidal epithelium on gastric cavity are smaller than the epithelial cell, more numerous and grouped (Table 2). This clustered shape increases the area of cell surface (Gartner and Hiatt 1997), which are specialized for different functions. In the case of the cilia of ctenidium, their function is to direct and generate respiratory currents in opposed directions of the flow of the blood within the ctenidial filament (Hughes 1986) and to maintain the water current that enters to the mantle cavity by the left side of the head and leaves by the right side (Hughes 1986, Warren 1959). In the case of the foot, the cilia serve to lead the secreted mucus for locomotion, protection, and feeding (Hughes 1986). In the case of the gastric cavity, the ciliary action of the rotating crystalline style directs the particles towards the stomach, the intestinal cavity, and the digestive gland (Warren 1959, Hughes 1986).

The secretions observed in the foot, penis, and ctenidium (Figures 4, 5, 7 and 9, respectively) according to Hughes (1986) are mucus that is important for the food capture, adhesion, locomotion, and to produce directional signs.

Gartner and Hiatt (1997) indicated that the loose connective tissue is characteristic of structures that do not require protection, being a characteristic

of tissues implied in the nutrition and the storage of proteins and fats. The fibrous connective tissue was only found in the proboscis, eye, antenna, and the anus, being structures that need support and expansion (Gartner and Hiatt 1997).

Table 1. Summary of the composition of epithelial tissue in eight structures of *Strombus gigas*

Structure	Epithelial type	Cilia length µm	Cell height µm	Position in the cell	Nuclei Size µm	Feature
Proboscis	Pseudo-stratified columnar	unciliated	60 – 80	middle	12 – 14 by 2 – 4	oval
Eye antenna	Pseudo-stratified columnar	unciliated	34 – 60	basal	9 – 12 by 2 – 3	oval
Foot	glandular columnar	8 – 10	40 – 48	basal	6 – 8 by 1 – 2	oval, granulose
	simple columnar	4 – 5	20 – 36	basal		rounded, granulose
	Pseudo-stratified columnar	4 – 5	36 – 40	basal		rounded, granulose
Ctenidium	simple columnar	30 - 40	20 – 28	middle	4 – 6	rounded, granulose
Penis	glandular	4 - 6	20 – 30			
Style sac	transi-tional	unciliated			4 – 6	rounded, granulose
	simple cuboidal	18 – 22	16 – 18	middle	6	rounded, granulose
Kidney	glandular		30	basal	7	
	simple columnar	unciliated	20 – 40	basal		
Anus	transitio-nal	unciliated				
	simple columnar	16 - 40	30 – 40	basal	2 – 8	oval

Table 2. Relation between of the cilia length *versus* epithelial cells height of the foot, penis, anus, ctenidium and style sac of the *Strombus gigas.*

Structure	Epithelial type*	Cilia length (CL) *versus* epithelial cells height (ECH)	Relation (CL : ECH) Cilia length *versus* epithelial cells height	Cluster of cilia
Foot	(1) (2) (3)	CL < ECH	1 : 6 (1); 1 : 8 (2); 1 : 5 (3)	grouped
Penis	(3)	CL < ECH	1 : 5	grouped
Anus	(1)	CL ≤ ECH	1 : 2 – 1 : 1	grouped
Ctenidium	(1)	CL ≥ ECH	1.5 : 1	separated
Style sac	(4)	CL ≥ ECH	approximately 1 : 1	grouped

* (1) simple columnar; (2) pseudostratified columnar; (3) glandular; (4) simple cuboidal.

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