

# STRUCTURAL ANALYSIS OF THE DIGESTIVE GLAND OF THE QUEEN CONCH *STROMBUS GIGAS* LINNAEUS, 1758 AND ITS INTRACELLULAR PARASITES

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## ABSTRACT

This study describes the structure of the digestive gland of *Strombus gigas* in individuals from Guadeloupe and discusses the function of its cell types and their relationship with intracellular Apicomplexa-like parasites. Three cellular types were found in the epithelium of the blind-ending tubules of the digestive gland according to histological and transmission electron microscopy (TEM) observations; these were: digestive cells, pyramidal crypt cells and vacuolated cells. Columnar digestive cells were characterized by large Alcian blue-positive granules, which have not been previously described in digestive cells of other caenogastropods. Such granules contain large quantities of proteoglycans that are exported to the stomach through the physiological destruction of the digestive cells, which undergo a holocrine secretion. Their cytoplasm appears vacuolar due to lipid extraction by solvents used for tissue preparation. Vacuolated cells also appear to be lipid-storage cells. Small triangular-shaped crypt cells, on the other hand, appear to be metabolically active as suggested by a strong positive *in situ* hybridization of eukaryotic ribosomes, which was confirmed by their large content of ribosomes and rough endoplasmic reticulum compared to the other cell types. These observations suggest that crypt cells may be immature cells that are involved in the replacement of eliminated digestive cells. However, their spherocrystal inclusions indicate that they may be excretory cells or calcium cells. Large brown inclusions were frequently observed in vacuolated cells; these were identified as parasitic protozoans and were present in the digestive gland of all sampled specimens. These protozoans have previously been described from a queen conch population in the San Andres Archipelago (Colombia). Several life cycle stages of the parasite were identified by scanning electron microscopy and TEM; trophozoites were characterized by their conoid-like structure, sporocysts by their thick walls, and gamonts by their thin walls. These observations suggest that this parasite completes its entire life cycle within the same host and type of tissue. Although previous investigations place this parasite within the Apicomplexa group, further investigations are necessary in order to confirm the identification of the parasite.

## INTRODUCTION

The queen conch, *Strombus gigas* Linnaeus, 1758, is a large caenogastropod mollusc that represents an important component of the marine fauna of the Caribbean Sea, not only because of its economic and cultural status, but also due to its ecological role as an algal grazer in seagrass beds and on coarse sand and gravel. Adult specimens possess a large crystalline style that measures up to 30 cm in length (Yonge, 1932a). The presence of a crystalline style is common in bivalves, but has only been described in four gastropod superfamilies; it has been proposed to be an anatomical feature associated with a continuous microphagous feeding habit (Yonge, 1932b; Graham, 1939; Fretter & Graham, 1962). Furthermore, some authors have reported a correlation between the presence of a crystalline style and the absence of salivary glands (Graham, 1939; Purchon, 1977). Nonetheless, there are many known exceptions to this latter pattern, and a necessary interdependence between the presence of a crystalline style, herbivory and a continuous feeding mode has recently been rejected (for review see Strong, 2003). For instance, although *S. gigas* has a crystalline style, Randall (1964) reported that this species is an

herbivorous grazer rather than a microphagous feeder and Little (1965) described large salivary glands for this species.

Little information at an histological level has been available about the digestive tract of this important species until recently (Avila Poveda, Aldana Aranda & Baqueiro Cardenas, 2005). Most studies of the digestive tract and digestive gland of microphagous and herbivorous gastropods have used species living in the intertidal environment to investigate the influence of tidal rhythms on the digestive gland cycle (Merdsoy & Farley, 1973; Bogen & Farley, 1974; Wigham, 1976; Nelson & Morton, 1979). Specifically, these investigations have been aimed at comparing the structure and function of the digestive gland in Bivalvia and microphagous Gastropoda. They have been synthesized for Caenogastropoda by Voltzow (1994), Rosenberg *et al.* (1997) and Strong (2003), for Pulmonata by Luchtel *et al.* (1997), for Bivalvia by Morse & Zardus (1997), and overall by Morton (1979) and Salvini-Plawen (1988).

Members of the superfamily Stromboidea are considered to be microphagous, because they presumably use their radulae to rake delicate filamentous algae or thin layers of organic material (i.e. biofilm) from blades of *Thalassia testudinum* or sand grains (Yonge, 1932b). However, several studies have reported individuals of *S. gigas* feeding on soft and hard macroalgae (Randall, 1964; Berg, 1974, 1975; Stoner & Waite,

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1991). Such feeding mode is very different to that reported for microphagous bivalve suspension- and deposit-feeders. The digestive gland tubules of bivalves, which form the basic model for microphagy in molluscs, are composed of two cell types that undergo cyclic modifications related primarily to food availability and processing (Owen, 1966; Pal, 1971, 1972; Henry, 1984a, b). The gastropod digestive gland, on the other hand, presents a more complex structure that suggests differences in diet composition and feeding mode compared to bivalves (Andrews, 1965; Lufty & Demian, 1967; Sumner, 1969; Walker, 1972; Merdsoy & Farley, 1973; Lopez, Gonzalez & Perez, 2003). Results from feeding trials using formulated pellets have raised new interest in the diet composition, as well as the structure and function of the digestive gland, of *S. gigas* (Aldana Aranda *et al.*, 2008). Furthermore, previous studies by Baqueiro Cardenas *et al.* (2007a, b) reported the presence of an intracellular protozoan parasite in the digestive gland of specimens of *S. gigas* in the San Andres Archipelago (Colombia). These parasites presumably belong to Apicomplexa and were found in all *S. gigas* specimens sampled throughout an entire year.

The main goal of this study was to describe the structure of the digestive gland in *S. gigas* at an histological and cytological level and to compare results with previous findings for other gastropods, especially herbivorous grazers and microphagous feeders. In addition, we were also interested to search for intracellular parasites in the digestive gland for another *S. gigas* population distant from Colombia.

## MATERIAL AND METHODS

### *Specimen collection and histological preparation*

*Strombus gigas* specimens were collected by skin diving at a depth of 20–24 m off the coast of the island of Guadeloupe (French Caribbean). Specimen collection was made possible thanks to a permit issued by the French Administration of Fisheries, which allowed the collection of 20 individuals per month, over a 16-month period. All sampled specimens were adults with shell lips over 6 mm thick and belonged to a single *S. gigas* population located at la Pointe des Chateaux on the east coast of Guadeloupe. Two samples of tissue were obtained from each specimen by cutting the sub-terminal portion of the visceral hump, and were immediately fixed in 10% neutral formalin in sea water and later in alcoholic Bouin's fixative. Subsequently, tissue samples were dehydrated in an ethanol series and Clarène, and then infiltrated with Paraplast wax before embedding. Wax sections, 6 µm-thick, were obtained from each sample and stained with a modified Goldner trichrome method that included Alcian blue at pH 2.5 in order to stain proteoglycans (Gabe, 1968).

Samples of faeces collected from adult specimens were mixed with sterile sea water and immediately observed, without being stained, under a light microscope.

### *Fluorescent in situ hybridization experiments*

Samples were fixed for 1–3 h at 4°C in 3% paraformaldehyde in 0.22 µm-filtered seawater. Samples were then rinsed three times for 10 min at 4°C in 0.22 µm-filtered seawater, and then dehydrated in an ascending series of ethanol and stored in 100% ethanol at 4°C until they were embedded in paraplast. Sections, 4 µm-thick, were placed on precoated slides from Sigma before hybridization. The oligonucleotide Cyanine3-labelled probe Euk1379 (5'-TACAAAGGGCAGG GAC-3'), directed against eukaryotic rRNA, was used. Oligonucleotidic *in situ* hybridization experiments were similar

to those previously described by Dubilier *et al.* (1995), with a hybridization time of 3 h at 46°C.

### *Electron microscopy*

*Transmission electron microscopy.* For ultrastructural observations, small pieces of adult digestive gland were fixed for 2 h in 3% glutaraldehyde in a 0.2 M cacodylate buffer at pH 7.2, which was made isosmotic to seawater by addition of sodium chloride. Samples were then rinsed with an isosmotic buffer and postfixed for 1 h in 1% OsO<sub>4</sub> in the same buffer, dehydrated in an ethanol series and propylene oxide, and finally embedded in an Epon-Araldite mixture. Semi-thin sections (0.5–1 µm-thick) were cut from the resin-embedded samples and stained with 0.5% toluidine blue in 1% borax for light microscopy observation. Thin sections (60 nm-thick) were cut for transmission electron microscopy (TEM) observation, processed and stained by uranyl acetate and lead citrate using standard TEM methods and observed in a Leo 912 TEM microscope.

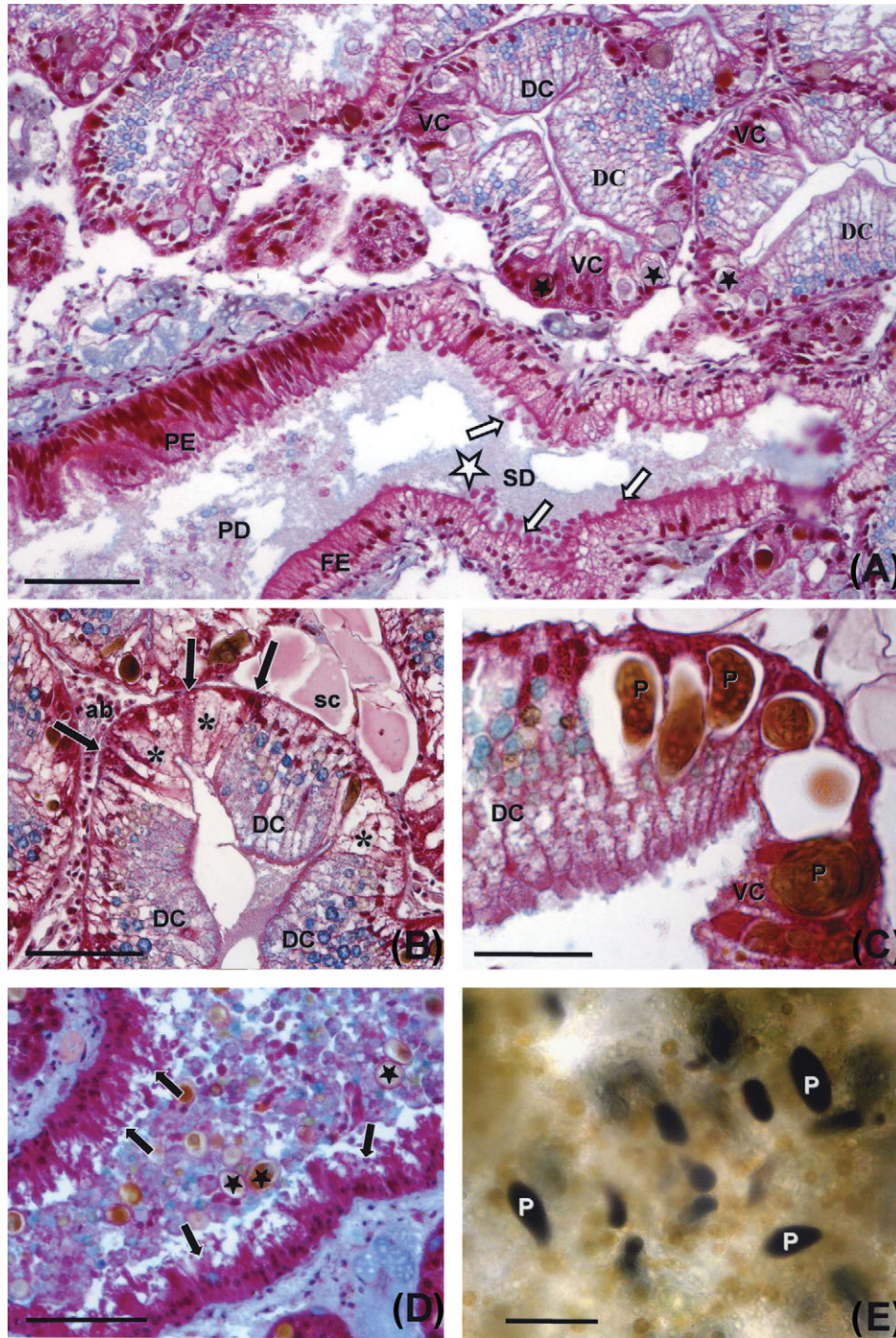
*Scanning electron microscopy.* In order to obtain a purified parasite-fraction, a piece of digestive gland was crushed in 6 ml of sterile seawater using a hand-held Dounce homogenizer. The homogenized tissue was centrifuged (30 g for 1 min), and 4 ml of the supernatant were then gently layered on top of a 50% percoll cushion (5 ml) diluted with imidazole buffered saline (490 mM NaCl, 30 mM MgSO<sub>4</sub>, 11 mM CaCl<sub>2</sub>, 3 mM KCl and 50 mM imidazole), and centrifuged (300 g for 4 min at 4°C). Parasite specimens were then collected from under the cushion, rinsed once, and suspended in 1 ml of 0.22 µm-filtered seawater to obtain a purified parasite suspension. Samples were fixed as described for the TEM preparation, and then dehydrated in acetone, critical-point dried and sputter-coated with gold before observation in a Hitachi 2500 scanning electron microscope.

## RESULTS

### *Histology of digestive gland*

The large dark brown digestive gland of *Strombus gigas* was covered by connective tissue that appeared translucent when individuals were immature and creamy white (female) or brick red (male) when the gonad was mature. It occupied most of the coiled visceral hump. The digestive gland was an array of blind tubules or acini connecting with small secondary ducts that joined to form two large primary ducts opening into the stomach. Primary and secondary ducts had a very different appearance based on histological sections (Fig. 1A). Large primary ducts were lined by two different groups of epithelial cells, some plicate portions composed of ciliated cells alternating with flat non-ciliated cells bordered by microvilli (Fig. 1A). Goblet cells stained by Alcian blue were interspersed with non-ciliated cells. Small secondary ducts were bordered by epithelial cells approximately 25 µm in height with central nuclei. Their apical poles were frequently drop-shaped with abundant emission of cellular components similar to apocrine secretion (Fig. 1A, D) generally observed at the apical pole of secreting cells rather than duct cells.

The digestive gland tubules were separated from the connective tissue lining their base by a distinct basal lamina (Fig. 1B). The tubules appeared to be composed of three cell types (Fig. 1B). The most abundant were narrow-columnar digestive cells, up to 100 µm in height and only 10 µm wide. These cells were packed together to constitute two large functional units facing each other in transverse sections (Fig. 1A, B). Each functional unit was up to 100 µm in width.



**Figure 1.** Histology of the digestive gland of *Strombus gigas*. **A.** Micrograph of a histological section of a portion of the digestive gland comprising several glandular portions and a section of the primary and secondary ducts. A large primary duct (PD) is bordered by two different epithelial structures: a plicate epithelium (PE) and a flat epithelium (FE) bordered by microvilli. A smaller secondary duct (SD) connects the glandular part with the primary ducts of the digestive gland. Its epithelium is characterized by abundant blebs (arrows) at the apical pole. These ducts contain coarse inclusions originating from the digestive cells and the secondary ducts, as well as a flocculent substance (white star) that may have originated from the stomach. Sections of the glandular portion are composed of large functional groups of tall digestive cells (DC) containing numerous large granules stained blue by Alcian blue and of smaller crypt areas stained mostly red containing vacuolated cells (VC) with large round inclusions (stars). **B.** Higher magnification micrograph showing the organization of the secretory region of the gland. Digestive cells (DC) appear long and narrow and make up a functional unit. The crypt area is occupied by two different cell types: large vacuolated clear cells (asterisks) interspersed with small pyramidal dark cells (arrows). The connective tissue is composed of large storage cells (sc) and small amoebocytes (ab). **C.** Large elongated parasites (P) occupy the digestive (DC) and vacuolated (VC) cells. **D.** Secondary duct full of cellular components derived from the holocrine secretion cycle of the digestive cells with typical blue granules; the duct epithelium takes part in the production of the digestive gland by apocrine emission (arrows). Large yellowish parasites (stars) are emitted when the digestive cells are destroyed. **E.** Smear of faeces containing numerous elongated parasites (P), which demonstrates that these protozoans travel from the digestive gland through the stomach and intestine, and are finally released with faeces. Scale bars: **A** = 50  $\mu$ m; **B** = 40  $\mu$ m; **C** = 10  $\mu$ m; **D** = 30  $\mu$ m; **E** = 20  $\mu$ m.

In addition, large granules of up to 10–12  $\mu\text{m}$  in diameter were stained by Alcian blue and were frequently observed occupying a large portion of these digestive cells, although in many cases they were restricted to the basal region of the cell (Fig. 1A–C). Some smaller granules that were stained bright red by acid fuchsin occasionally occupied the apical pole of these digestive cells. Such cells undergo a physiological cycle of destruction; their apical pole showed blebs that were released into the gland's lumen through an apocrine or holocrine secretion process (Fig. 1A). The export of typical large blue granules to the duct system was clearly seen (Fig. 1D).

Groups of columnar digestive cells alternated with crypt regions composed of two other cell types (Fig. 1B). The larger and most conspicuous type appeared as clear vacuolated cells up to 50  $\mu\text{m}$  in height with small basal nuclei. Their cytoplasm was stained by acid fuchsin or any other general cytoplasmic stain. Vacuolated cells were interspersed with small, slender pyramidal basophilic cells (Fig. 1B) that were stained by haematoxylin and cytoplasmic dye. Each of the pyramidal cells, which we named crypt cells, had a central nucleus with a conspicuous nucleolus. These pyramidal cells contained some small inclusions (less than 2  $\mu\text{m}$  in diameter) characterized by an Alcian blue-positive central granule. Crypt cells were positive to hybridization with fluorescent *in situ* hybridization (FISH) ribosome labelling, which demonstrates that they are actively involved in protein synthesis (Fig. 2). This labelling did not include crypt cells; large vacuolated cells and digestive cells were negative to oligonucleotidic *in situ* hybridization.

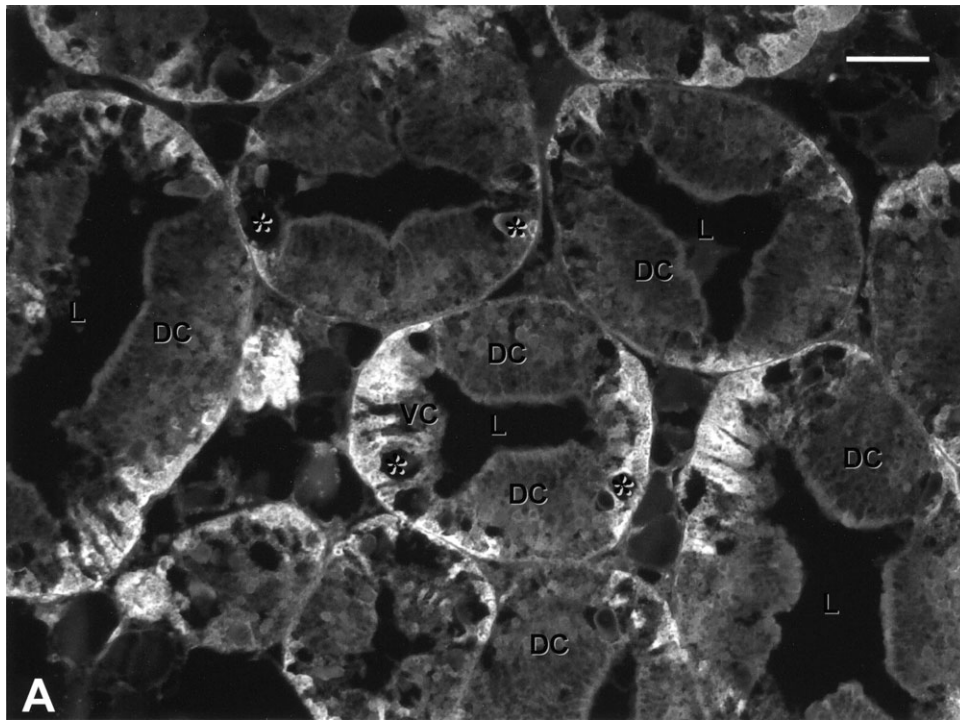
The contents of primary and secondary digestive ducts appeared to be of different origins. While digestive cell secretions travel from the digestive gland to the stomach, demonstrated by the presence of excreted blue granules, the presence of a contrasting finely grained grey particulate substance in the primary and secondary ducts (Fig. 1A) may

result from the circulation of digested food that may be absorbed by the digestive cells. Nonetheless, there was no evidence of intracellular digestion in this latter case.

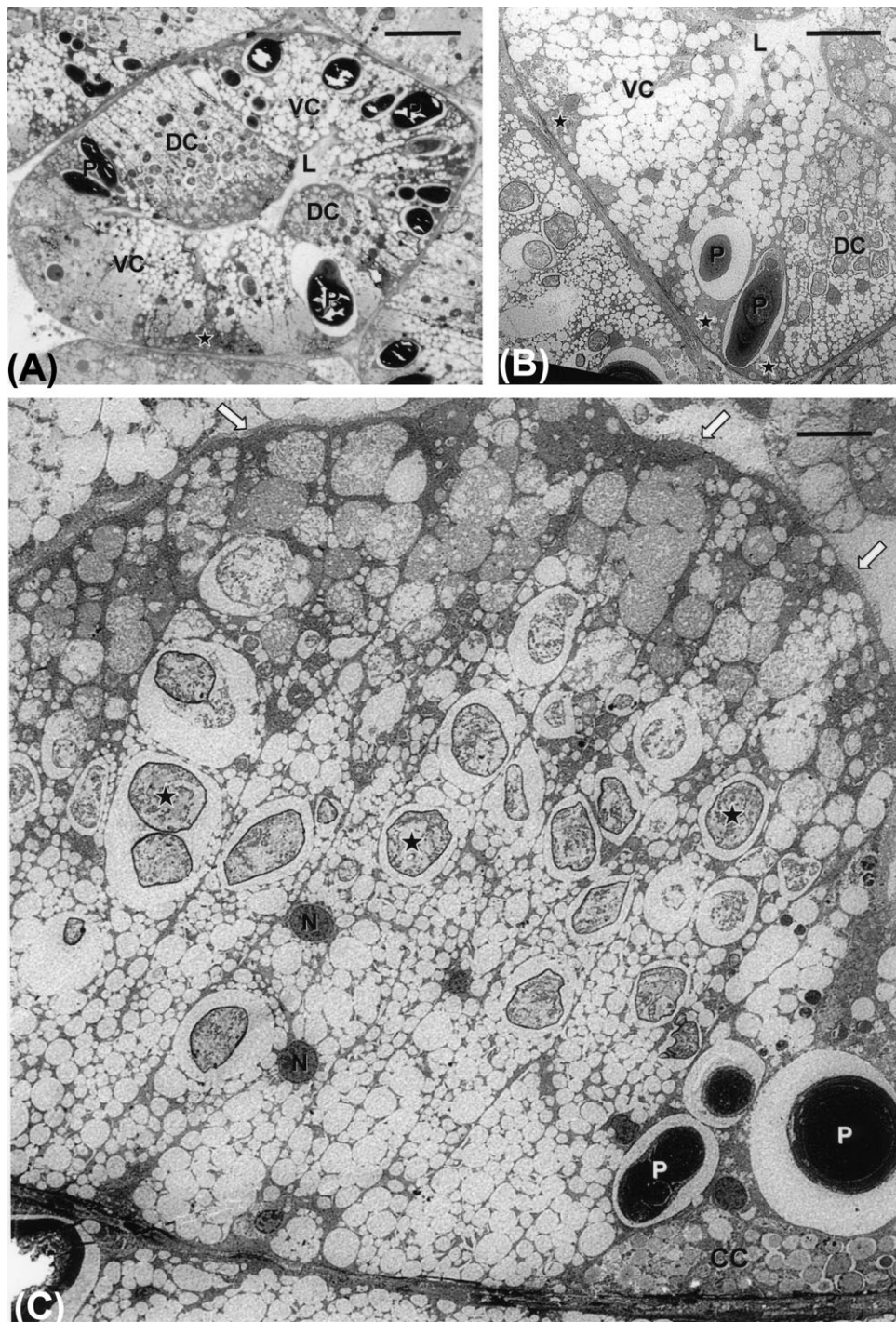
*Intracellular parasites in histological sections.* Brown inclusions were frequently found in vacuolated cells; these inclusions reached up to 35  $\mu\text{m}$  in width when elongated and 25  $\mu\text{m}$  in diameter when of a more rounded shape (Fig. 1C). However, it is difficult to determine if their shape actually varies or if these differences were only an artefact of the orientation of the section. Observations of unstained sections demonstrated that the brown or black colour of these inclusions is natural.

Shapes and structures such as narrow bottle shapes resembled the typical trophozoite stage of protozoan parasites. Round stages with a thick envelope were identified as sporocysts, whereas others with a thin envelope were identified as gamonts. These inclusions were less frequent in the digestive cells and completely absent from pyramidal crypt cells. They were also observed in the lumen of the digestive gland ducts (Fig. 1D) and in faeces (Fig. 1E) where their shape was not modified by sectioning.

*Ultrastructure of digestive gland cells.* The three digestive gland cell types were clearly distinguished in the semi-thin sections (Fig. 3A) and were also characterized by their inclusions observed in TEM sections. The cytoplasm of the tall and narrow digestive cells had vacuoles which were much smaller than those observed in the vacuolated cells (Fig. 3B). The large inclusions (8–10  $\mu\text{m}$  in diameter) identified histologically by Alcian blue appeared as vacuoles containing dense heterogeneous granules (Figs 3C, 6A). These inclusions did not look like lysosomal residual bodies and their function is unknown. The apical area of the digestive cells was occupied by two



**Figure 2.** Fluorescent *in situ* hybridization using universal probe for 18S rRNA, revealing eukaryotic ribosomes. Light micrograph. Strong positive hybridization of crypt cells (appearing white) due to a large content of ribosomes. Vacuolated (VC) and digestive (DC) cells are devoid of a positive signal suggesting a relatively low content of ribosomes in their cytoplasm. Parasites (asterisks) are also not hybridized by the eukaryotic universal probe. Scale bar = 150  $\mu\text{m}$ .

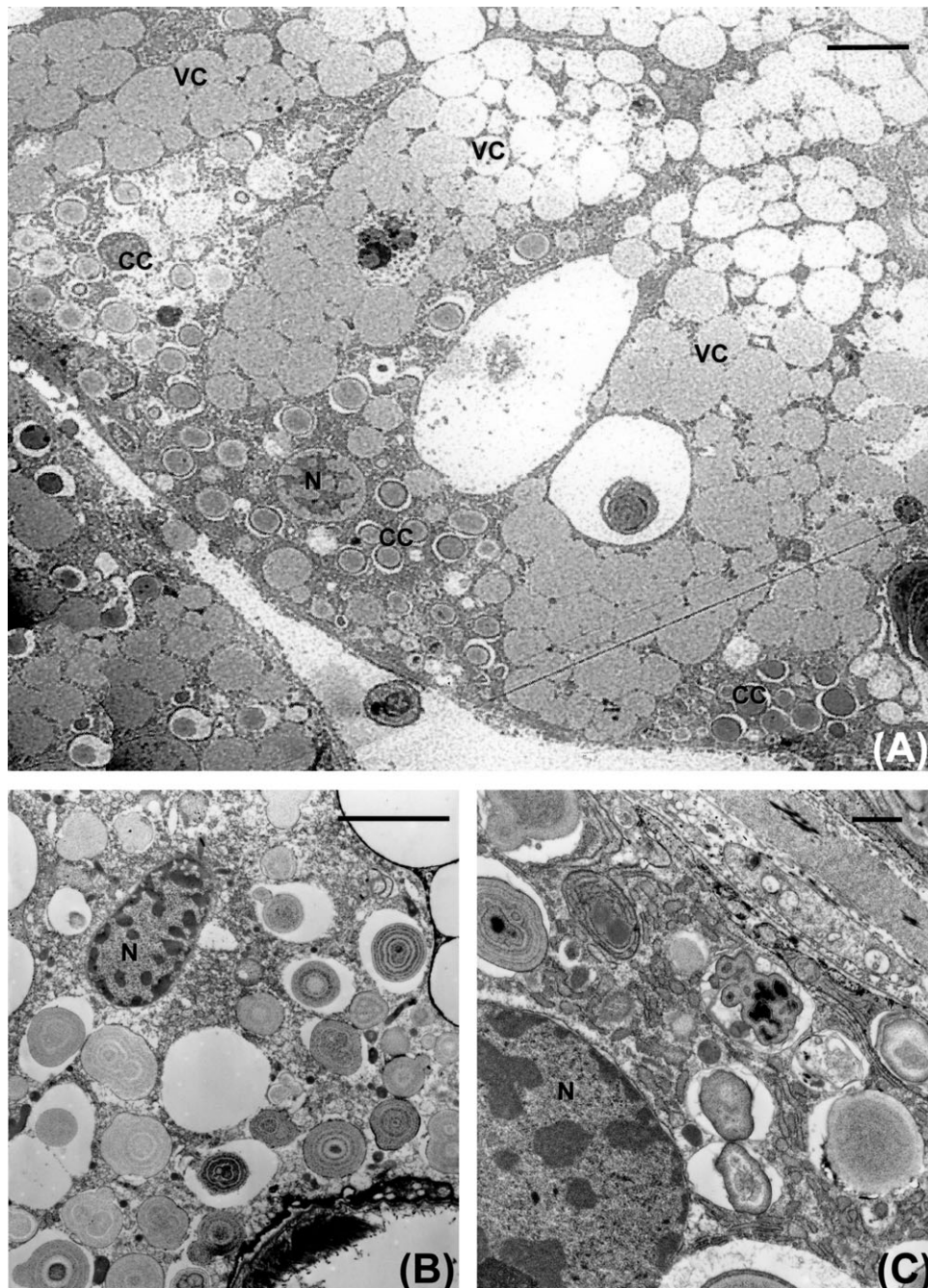


**Figure 3.** Structure of the digestive gland of *Strombus gigas*, viewed by light microscopy and TEM. **A.** Semi-thin section of an acinus from the digestive gland of *S. gigas*. Parasites (P) appear to be very hard due to their thick envelopes and were frequently damaged by sectioning. Parasites are located primarily in vacuolated cells (VC), while digestive cells (DC), characterized by their large size and numerous granules, do not harbour such parasites. A third cell type, corresponding to crypt cells, is indicated by stars. **B.** Low magnification TEM view of the basal portion of an acinus showing envacuolated parasites (P) located between digestive and vacuolated cells. **C.** TEM of digestive cells with numerous large envacuolated heterogeneous inclusions corresponding to those that were positively stained by Alcian blue in histological sections (stars). The rounded apical pole is covered by short microvilli (large arrows). Other abbreviations: L, lumen of the acinus; N, nucleus; P, parasite. Scale bars: **A** = 50  $\mu\text{m}$ ; **B** = 30  $\mu\text{m}$ ; **C** = 10  $\mu\text{m}$ .

different types of inclusions (Fig. 3C). Some large grey inclusions may represent a maturation stage of the blue granules, whereas small clear ones correspond to the small inclusions stained histologically by acid fuchsin and may be enzymatic secretions (Fig. 3C). The apical pole of the digestive

cells was bordered by short microvilli (Fig. 3C). We have not yet observed endo- or exocytosis in our images.

Vacuolated cells in TEM sections were characterized by large clear vacuoles that may be lipid storage inclusions (Fig. 4A). These inclusions appeared grey at the base of the



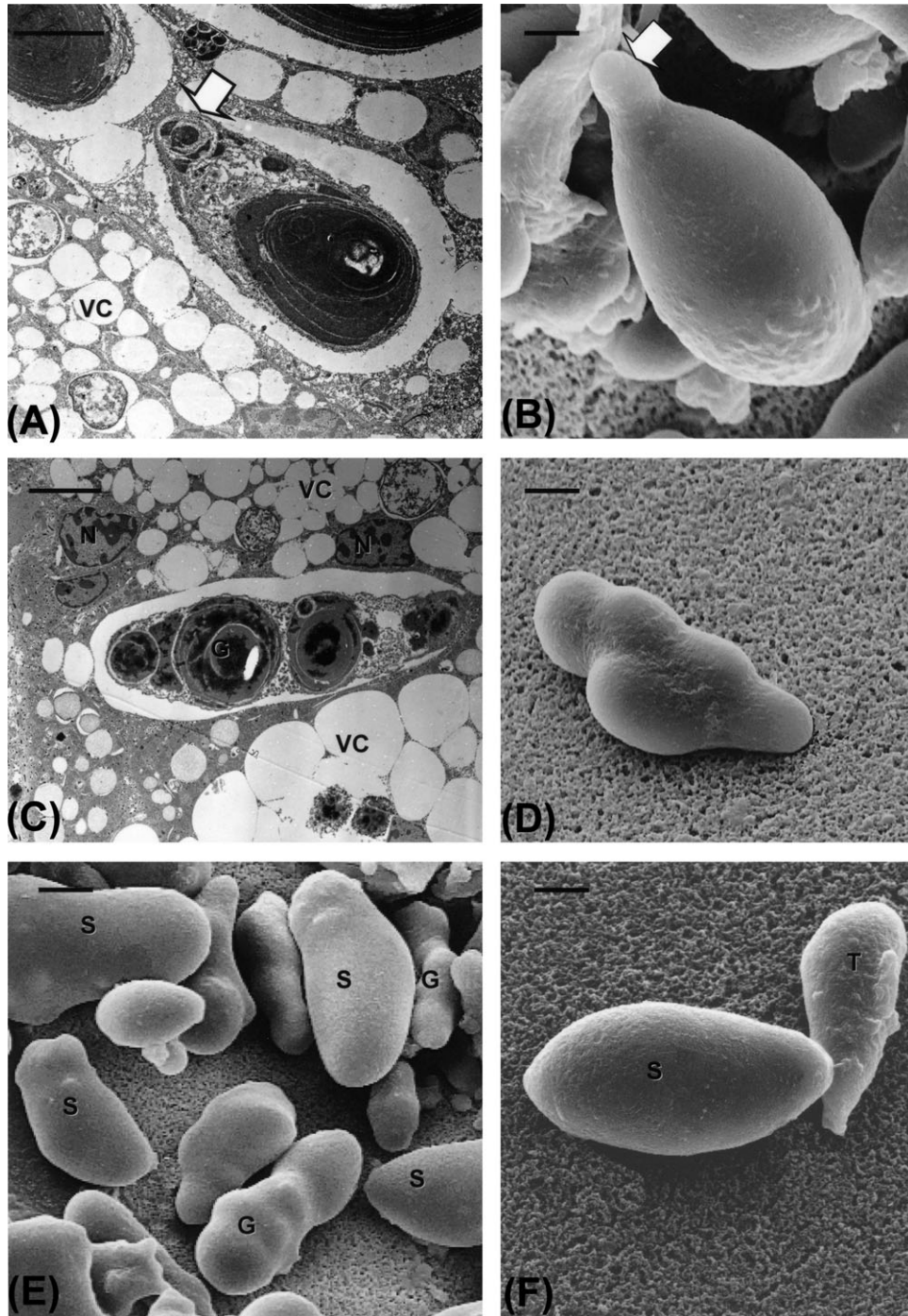
**Figure 4.** Digestive gland cells of *Strombus gigas* viewed by TEM. **A.** Pyramidal crypt cells (CC) are characterized by large basal poles and narrow apical poles. Their cytoplasm contains numerous small inclusions. Vacuolated cells (VC) are characterized by a cytoplasm filled with large vacuoles probably serving for lipid storage. **B.** Higher magnification TEM of the cytoplasm of a crypt cell. Each small inclusion, located in vacuoles that may be slightly widened, is characterized by several concentric layers similar to concentrically layered spherocrystals common in excretory cells. **C.** The cytoplasm of crypt cells is also characterized by an extensive RER distributed between the spherocrystal-like inclusions. Other abbreviation: N, nucleus. Scale bars: **A** = 10  $\mu\text{m}$ ; **B** = 5  $\mu\text{m}$ ; **C** = 1  $\mu\text{m}$ .

cells and clear in the upper regions (Fig. 4A); such differences correspond to an apparent metachromatic basal area observed in histology. However, electron microscopy did not reveal any new features.

The third and smallest type of cell was observed between the previously described cell types or interspersed between the vacuolated cells. Cells belonging to this cell type were named crypt cells, and were characterized by having an extensive rough endoplasmic reticulum (Fig. 4C), corresponding to the

basophilic histological staining and the positive FISH-labelling of ribosomes observed in light microscopy. These crypt cells also contained small concretions of less than 2  $\mu\text{m}$  in diameter (Fig. 4A–C), characterized by having concentric circles too small to be identified with light microscopy. These structures are typical of spherocrystalline inclusions.

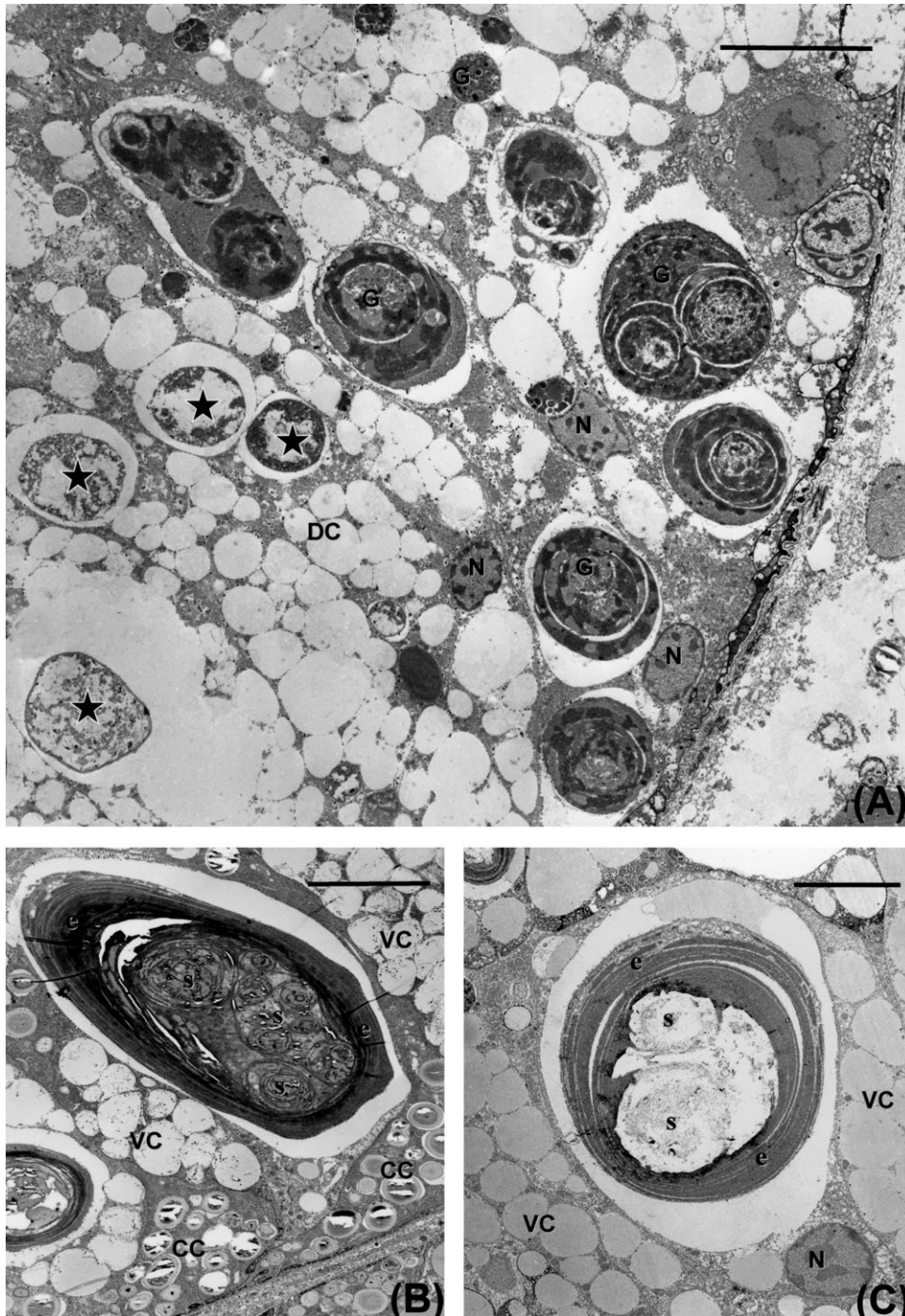
*Ultrastructure of parasites.* Large dark inclusions considered to be parasites were found most frequently in vacuolated cells. Some



**Figure 5.** SEM of various life cycle stages of a purified parasite fraction of the digestive gland of *Strombus gigas* compared to TEM *in situ* sections of these same stages. **A.** TEM of a trophozoite stage with a conical structure (large arrow). This stage is characterized by a thin wall and internal structures limited by several whorls of envelopes. **B.** SEM view of a similar bottle-shaped trophozoite stage. The conoid (large arrow) is characteristic of the trophozoite stage. **C.** TEM of a gamont, characterized by a thin envelope containing several rounded inclusions that may be macrogametes. This gamont (G) is between two vacuolated cells (VC). **D.** SEM view of a gamont stage from a purified parasite fraction. The internal inclusions form obvious protrusions typical of gamonts. **E.** SEM of mixed stages from a purified parasite fraction of *S. gigas*. Gamonts (G) are characterized by their external protrusions whereas sporocysts (S) appear larger with smooth surfaces. **F.** SEM of two stages, trophozoite (T) and sporocyst (S), observed from a purified parasite fraction of *S. gigas*. Other abbreviations: N, nucleus; VC, vacuolated cell. Scale bars: **A** = 2  $\mu\text{m}$ ; **B** = 5  $\mu\text{m}$ ; **C** = 5  $\mu\text{m}$ ; **D** = 10  $\mu\text{m}$ ; **E** = 20  $\mu\text{m}$ ; **F** = 10  $\mu\text{m}$ .

of these parasites were also observed between the digestive cells and vacuolated cells, rather than within the digestive cells (Fig. 3B, C). Observations with TEM and scanning electron microscopy (SEM) suggested three different life-cycle stages for these parasites. First, trophozoites were large bottle-shaped

organisms with a conical structure (Fig. 5A) identified with SEM (Fig. 5B) and an internal structure of several thick envelopes observed with TEM (Fig. 5A). Another stage, characterized by a thin envelope and containing several rounded inclusions (Figs 5C, D, 6A) was considered to be a gamont



**Figure 6:** *In situ* characterization of parasite stages in the digestive gland of *Strombus gigas*. **A.** Several gamonts (G) located in a vacuolated cell adjacent to a digestive cell (DC) characterized by its typical large granules (stars). Each gamont has a thin envelope overlying internal structures characterized by concentric layers. **B.** TEM of a sporocyst characterized by a thick envelope (e) surrounding several inclusions that may be spores (s). **C.** Envacuolated sporocyst with its thick envelope (e) consisting of several concentric layers. The envelope surrounds and protects the inner spores (s), which are poorly fixed due to the impenetrability of the thick envelope. Other abbreviations: CC, crypt cell; N, nucleus; VC, vacuolated cell. Scale bars: **A** = 10  $\mu\text{m}$ ; **B** = 10  $\mu\text{m}$ ; **C** = 5  $\mu\text{m}$ .

stage that produces macro- or microgametes. Finally, the most common parasite stage appeared as elongated smooth structures devoid of conical structure (Fig. 5E, F). This stage may correspond to sporocysts characterized by thick and hard envelopes; such sporocysts contained several macro- or microspores (Fig. 6B, C).

## DISCUSSION

A review by Voltzow (1994) summarizes research on the digestive gland of Caenogastropoda, including several cases of histological and ultrastructural descriptions according to feeding type (microphagous, herbivorous or carnivorous). Those



Caenogastropoda that possess a crystalline style have been considered to be microphagous with intracellular digestion and to show absorption similar to that of bivalves (Yonge, 1932a; Graham, 1939). *Strombus gigas* possesses a crystalline style and belongs to the superfamily Stromboidea, which is considered to be microphagous. However, members of this species have been shown to feed on several macroalgae according to food availability (Randall, 1964; Berg, 1974, 1975; Stoner & Waite, 1991). The digestive gland of *S. gigas* appears to be more complex compared to those described for other microphagous, herbivorous or carnivorous gastropods. One exception is that of *Aplysia punctata* (Taïeb & Vicente, 1999; Taïeb, 2001) which shows two different types of digestive tubules with distinct cell types, including special calcium cells in one type of tubule (tubule A) which do not occur in a second type of tubule (tubule B). In the case of *S. gigas*, a differentiation of tubule types A and B is not obvious, because crypt cells containing calcium sphaerules similar to those described by Taïeb & Vicente (1999) were observed in all tubule sections.

Special granules that stained positively with Alcian blue and were observed in all digestive cells in *S. gigas* may be similar to the cyanophilic vesicles that have been described in the B1 digestive tubules of *A. punctata* (Taïeb, 2001). Such granules may be secretory products, because they are regularly found in the lumen of the digestive ducts in *S. gigas*. Such conspicuous secretory granules serve as markers to demonstrate that secretions from the digestive cells are being delivered to the stomach. Nonetheless, the exact function of these granules remains unknown. Cells containing such blue granules in *S. gigas*, first described as 'secretory cells' by Baqueiro Cardenas *et al.* (2007b), are named 'digestive cells' in the present study. However, based on our ultrastructural analysis we have modified the nomenclature of the other two cell types composing the digestive tubules in *S. gigas*. Cells previously described as 'crypt cells' are now named 'vacuolated cells', while the small pyramidal cells (not described by Baqueiro Cardenas *et al.*, 2007b) are what we call 'crypt cells', following Taïeb & Vicente (1999). Additional findings from this study demonstrate the secretion by digestive cells of abundant proteoglycans, quite distinct from ordinary mucous secretion; such granules may be involved in detoxification by elimination of sulphide components. However, no evidence of intracellular digestion was detected in digestive cells, which lack typical residual lysosomal bodies. Therefore, digestion appears to take place extracellularly inside the stomach, and finely ground material coming from the stomach may be digested food intended for absorption by the digestive gland cells. However, we have no evidence of which cell type is involved in the absorption of digested materials.

Digestive and vacuolated cells show a vacuolar cytoplasm consistent with abundant lipid storage. Staining of cryosections with Sudan black demonstrated that the basal portion of these two cell types contained abundant lipid droplets (Volland, unpublished data). Therefore, vacuolated cells can be considered as storage cells in *S. gigas*. The small pyramidal crypt cell, on the other hand, had a characteristic structure as revealed by electron microscopy. Their rough endoplasmic reticulum may indicate young secretory cells in an early stage of differentiation. However, the presence of sphaerocrystals is not a feature of immature cells. Crypt cells may thus be involved in mineral storage, as well as in the metabolism of calcium and magnesium (Thomas *et al.*, 1999) or in ionic regulation. Pyramidal crypt cells are apparently absent from the digestive gland of juvenile specimens either collected in the field or used in an artificial diet experiment (Aldana Aranda *et al.*, 2008). More information is necessary to determine the precise function of these cells, as well as to identify what other cell types may replace destroyed digestive cells.

Large brown inclusions also occurred frequently in vacuolated cells and to a lesser extent in digestive cells. Such inclusions have previously been described as Apicomplexa-like organisms (Baqueiro Cardenas *et al.*, 2007a, b). The taxon Apicomplexa (Perkins, 1991) contains 5,000 species, all of which are considered to be protozoan parasites. So, based on the structures observed with SEM and TEM (presence of an apical complex), we consider these parasites to be members of the Apicomplexa. When digestive cells are infected, the parasites take advantage of the digestive cell's physiological cycle to disperse outside the organism via faeces, although they do not contribute to cellular destruction. The large number of parasites observed in each digestive gland of *S. gigas* from Guadeloupe agrees with previous findings from another population sampled from San Andrés Island in Colombia (Baqueiro Cardenas *et al.*, 2007b). However, the life cycle of such parasites has not yet been described.

The presence of Apicomplexa-like protozoans was observed in all of the *S. gigas* specimens sampled from Guadeloupe throughout the year, including both males and females. Parasite presence was much more frequent in vacuolated cells, but may also occur in digestive cells. Interestingly, in those cases for which vacuolated cells were the only cell type infected, the connecting ducts did not contain parasites; in contrast, when the digestive cells were infected, parasites were observed in both primary and secondary connecting ducts. This finding suggests that parasites are delivered to the stomach and gut when present in this latter cell type. Given that various stages of the parasite were observed in the digestive gland of *S. gigas* specimens, we suspect that the entire life cycle of this parasite is completed within the same host species and tissue. In addition, parasites were observed in freshly deposited faeces, which probably serves for dispersal and subsequent infection of other individuals of the same species or other species living in the same habitat.

Apicomplexan infection has been previously described in several orders of marine gastropods including Vetigastropoda (Friedman *et al.*, 1995; Azevedo *et al.*, 2006) and Neritoida (Azevedo *et al.*, 2004), besides the caenogastropod Stromboidea (this study). A similar type of infection has also been described in the digestive gland of the filibranch bivalve *Crassostrea commercialis* (Bower *et al.*, 1994) in which digestive gland tubules are massively infected by various stages of an apicomplexan parasite belonging to Paramyxea (Bower *et al.*, 1994). However, the outcome of this infection in *C. commercialis* was very different from that observed in *S. gigas*, as the digestive gland tubules of this bivalve were completely disorganized resulting in the death of the infected specimens.

Additional research is in progress in order to understand the structure and function of each cell type found in the digestive gland of *S. gigas*, as well as to understand the relationship between the digestive system and its associated Apicomplexa-like protozoan parasite.

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