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Journal of
Molluscan Studies

The Malacological Society of London

Journal of Molluscan Studies (2020) **0**: 1–9. doi:10.1093/mollus/eyaa026

Ontogenesis of the digestive gland through the planktotrophic stages of *Strombus gigas*

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(Received 30 June 2019; editorial decision 28 April 2020)

ABSTRACT

The queen conch, *Strombus gigas* (Linnaeus, 1758), is a marine mollusc of ecological and economic importance in the Caribbean. Its populations are declining due to overexploitation. We describe ontogenesis of the digestive gland in *S. gigas* during the larval stages. Larvae were studied over a period of 42 d in laboratory culture, from eggs to crawling stage. Experiments were conducted at 28 ± 1°C. Veligers were reared at a density of 100 larvae l^{-1} in 10-l containers. Larvae were fed with the microalgae *Nannochloropsis oculata* at a concentration of 1,000 cells l^{-1}. In this study, we analysed ultrastructural ontogenesis of the digestive gland in strombid larvae using light and electron microscopy. Examination for Coccidia (Apicomplexa) symbionts in the digestive gland was done by viewing sections with light and scanning electron microscopes at different larval development stages during a 42-d period. In early veligers (9 d after hatching), only digestive cells were observed in the digestive gland. By the late veliger stages (17 d old), both digestive and crypt cells were apparent in the digestive gland. Within crypt cells, spherocrystals were detected and the presence of Ca, Cl, Cu, P and Zn was identified by energy-dispersive X-ray spectroscopy. In late pediveligers (35 d old), the digestive gland still exhibited only digestive and crypt cells. Vacuolated cells (i.e. harbour the coccidian symbionts in adults) were only observed in newly settled juveniles (42 d old) and were devoid of apicomplexan structures. While coccidian symbionts were found in the digestive gland of adult *S. gigas*, they were not observed in the digestive gland of *S. gigas* larvae from hatching to settlement under laboratory conditions. This suggests that this symbiont is not vertically transmitted to new host generations in this marine gastropod species.

INTRODUCTION

The queen conch, *Strombus gigas* (Linnaeus, 1758), is an ecologically and economically important gastropod mollusc in the Caribbean. The critical state of its populations (Appeldoorn, 1992; Aldana Aranda, Baqueiro & Mazanilla, 2003a; Aldana Aranda *et al.*, 2003b) is driving research into its reproductive biology to support management decisions (Delgado *et al.*, 2004; Frenkiel *et al.*, 2009; Aldana Aranda *et al.*, 2014). In these reproductive studies, an Apicomplexa-like parasite was first detected in histological sections of the digestive gland of adult populations of *S. gigas* on San Andres Island, Colombia (Baqueiro *et al.*, 2007). Ultrastructural studies confirmed the presence of Apicomplexa-like parasites in the cytoplasm of vacuolated cells in the digestive gland (Gros, Frenkiel & Aldana Aranda, 2009; Volland *et al.*, 2010, 2012; Aldana Aranda *et al.*, 2011). Now known to be a coccidian, its presence in the digestive gland of *S. gigas* has been documented in various locations in the Caribbean (Aldana Aranda *et al.*, 2014). Apicomplexa are quite common in several molluscan species. They have been reported in bivalves such as *Ostrea edulis* (Azevedo, Montes & Corral, 1999); gastropods such as *Nerita ascensionis*; other gastropods including *Laevistrombus*, *Canariumurceus volutes* and *Nassarius* (Azmi, Ghaffar & Cob, 2019); abalones (Azevedo & Padovan, 2004); and cephalopods (Gonzalez *et al.*, 2003). Strombid gastropods, such as *S. gigas*, have been the focus of research since their digestive gland is known to host putative symbionts belonging to the Sporozoa group (Baqueiro *et al.*, 2007; Gros *et al.*, 2009; Volland *et al.*, 2010, 2012; Aldana Aranda *et al.*, 2011). However, no data are available concerning the ontogenesis of the digestive gland in strombid species during their larval life. The organogenesis and histogenesis of planktotrophic opisthobranch veligers of the nudibranch *Doridella steinbergae* (Bickell & Chia, 1979; Bickell, Chia & Crawford, 1981) helped to elucidate how the rudiments of benthic stage structures of apicomplexans develop within the veligers without impeding the processes of swimming and feeding. The ultrastructural analysis presented here provides an opportunity to determine when the host cells harbouring the symbionts in the digestive gland of strombids

appear in development and whether larvae reared in laboratory conditions harbour the apicomplexan symbionts.

A number of reviews have synthesized literature on the digestive glands of microphagous and herbivorous caenogastropods (Voltzow, 1994; Rosenberg *et al.*, 1997; Strong, 2003; Volland *et al.*, 2010). Members of the superfamily Stromboidea are considered to be microphagous. They presumably use their radulae to rake delicate filamentous algae or thin layers of organic material (i.e. biofilm) from blades of the marine seagrass *Thalassia testudinum* and from rocks, corals or sand grains. Compared to that of bivalves, the digestive gland of microphagous gastropods exhibits a more complex structure, suggesting differences in diet composition and feeding mode. The digestive gland of adult *S. gigas* appears even more complex than those described for other microphagous, herbivorous or carnivorous gastropods (Gros *et al.*, 2009). A study of veligers belonging to different superfamilies reveals the uniformity of structure of the alimentary tract, including the digestive diverticula up to the time of metamorphosis (Fretter & Montgomery, 1968). Development of the planktotrophic veliger of the dorid nudibranch *D. steinbergae* suggests that interspecific differences in the kinds of structures that develop during the veliger stage may relate to variations in the requirements of the juvenile phase (Bickell & Chia, 1979).

Histological and transmission electron microscopy (TEM) observations have helped to identify three cell types in the epithelium of the blind-ending tubules of the digestive gland: digestive cells, pyramidal crypt cells and vacuolated cells. The lysosomal enzymes acid phosphatase and arylsulphatase have been detected in the digestive cells, suggesting the occurrence of intracellular digestion within the digestive gland (Volland & Gros, 2012). Pyramidal crypt cells have spherocrystal inclusions that are involved in regulation of minerals and essential trace metals, but not in detoxification of nonessential trace metals (Volland & Gros, 2012). Crypt cells may be involved in mineral storage and metabolism of calcium and magnesium (Thomas, Stender-Seidel & Ckeler, 1999), or perhaps in ionic regulation. Vacuolated cells appear to have a role in lipid storage (Volland *et al.*, 2012), although in various strombids they also contain large brown inclusions identified as coccidian symbionts (Gros *et al.*, 2009; Volland *et al.*, 2010).

The organization of the digestive gland is well studied in adult strombids but little is known about its structure in larvae. A recent study of the planktotrophic larval development of *S. pugilis* (Enriquez-Diaz *et al.*, 2015) has described the morphological characteristics of the main developmental stages from early veligers to crawling juveniles based on light microscopy and scanning electron microscopy (SEM) images. Particular attention was given to the velum, shell whorls, the presence of a proboscis, foot and propodium, swimming and crawling behaviour and settlement, but no data were gathered on organogenesis.

The objective of the present study is to use light microscopy, SEM, and scanning transmission electron microscopy (STEM) to describe larval development of *S. gigas* from hatching to metamorphosis, and to describe the ontogenesis of the digestive gland that harbours Apicomplexa-like symbionts in the adult. This research constitutes the first description of the ultrastructure of ontogenesis of the digestive gland in strombids.

MATERIAL AND METHODS

Egg masses and reared larvae

The fertilized egg masses used in the experiments were collected in the waters of the Yucatan Peninsula, Mexico (22°21′N, 89°49′W). Egg masses were collected by scuba diving at 4 m depth, and only those in proximity to a female were collected to ensure species identity and freshness of the egg mass. Masses were immediately placed in an isothermic temperature container with seawater for transport to the laboratory. Macroscopic epibionts and sand particles were manually removed from the egg masses before washing with filtered and UV-sterilized seawater. Individual egg masses, from individual parent conchs, were each placed in a 300-μm mesh bag and kept immersed in a 25-l aquarium filled with 1 μm-filtered UV-sterilized seawater. Egg masses and larvae were reared from hatching to settlement as described previously (Aldana Aranda & Brito Manzano, 2017). Rearing conditions were water temperature of 28 ± 0.19 °C controlled with a diurnal plant growth chamber (SRI21D SHEL LAB) and a 12 h:12 h light:dark photoperiod.

After hatching, larvae were divided into three batches (each treated as a replicate) and reared in 10-l containers at a density of 100 larvae l^{-1}. Each batch of larvae was fed equal amounts of axenic fresh culture of the microalgae *Nannochloropsis oculata* at a 1,000 cell ml^{-1} concentration (Garcia Santaella & Aldana Aranda, 1994; Brito Manzano, Aldana Aranda & Baqueiro, 1999). Every 2 d, veligers were transferred to new containers with fresh 1 μm-filtered seawater. No chemical inducer was used to obtain fully metamorphosed juveniles 42 d after hatching.

Ten larvae were randomly collected for each major stage of larval development (Brito Manzano *et al.*, 1999; Enriquez-Diaz *et al.*, 2015; Chávez-Villegas, Enriquez-Diaz & Aldana Aranda, 2017) including veligers, pediveligers and plantigrades through 42 d of development. Larval growth was quantified by recording increases in the shell length axis ($n = 30$). Larvae were measured using a light microscope with a calibrated ocular micrometer (accuracy = 0.10 μm). Growth rate was calculated as average growth rate in μm d^{-1}.

Organogenesis

Characteristics of larval development were examined using light microscopy and SEM. Larvae were classified chronologically into early veligers (from hatching to 9 d old), late veligers (10–17 d old), early pediveligers (18–30 d old), late pediveligers (31–35 d old) and newly settled juveniles, with resorption of velar lobes and presence of crawling behaviour (42 d old) (Enriquez-Diaz *et al.*, 2015). Structural analysis of the digestive gland was carried out by randomly collecting ten larvae per batch from each larval stage and placing them in polyethylene specimen containers (Agar Scientific Co.) with a 60-μm mesh placed in the bottom to retain the larvae, for safe handling during relaxation and fixation. Specimens were anaesthetized for 5 min using a solution of 3 mM $MgCl_2$ in seawater before fixing for SEM and semithin examination. In preparation for SEM, larvae were fixed at 4 °C for 1 h in 2.5% glutaraldehyde in 0.1 mol l^{-1} cacodylate buffer adjusted to pH 7.2. After being rinsed in the same cacodylate buffer, larvae were dehydrated in an ascending series of acetone dilutions, and critical-point dried using CO_2 as the transitional fluid. For STEM and semithin sections, larvae were prefixed for 1 h at 4°C following the same procedure as described for SEM. They were then fixed for 45 min at room temperature in 1% osmium tetroxide in the same buffer and rinsed in distilled water. No decalcification process was done. Larvae were postfixed in 2% aqueous uranyl acetate for 1 h at room temperature before embedding in epoxy resin, following Mollenhauer (in Glauert, 1975). Semithin sections (0.5 μm thick) were cut using an ultramicrotome (Ultracut E Leica), mounted on clean slides on a hot plate (90 °C) and stained with 0.5% toluidine blue in 1% borax buffer; images were taken using a light microscope (Nikon Eclipse 80i). Ultrathin sections (60 nm thick) were cut and four to five sections examined using an electron microscope (FEI Quanta 250) at 20 kV in STEM mode. The STEM images presented here are representative of all the examined larvae.

Energy-dispersive X-ray spectroscopy

Detection of elemental compounds from spherocrystals was done by observing thin sections supported by nickel grids in an SEM instrument (FEI Quanta 250) at 20 kV in STEM mode. Energy-dispersive X-ray spectroscopy (EDXS) of individual spherocrystals

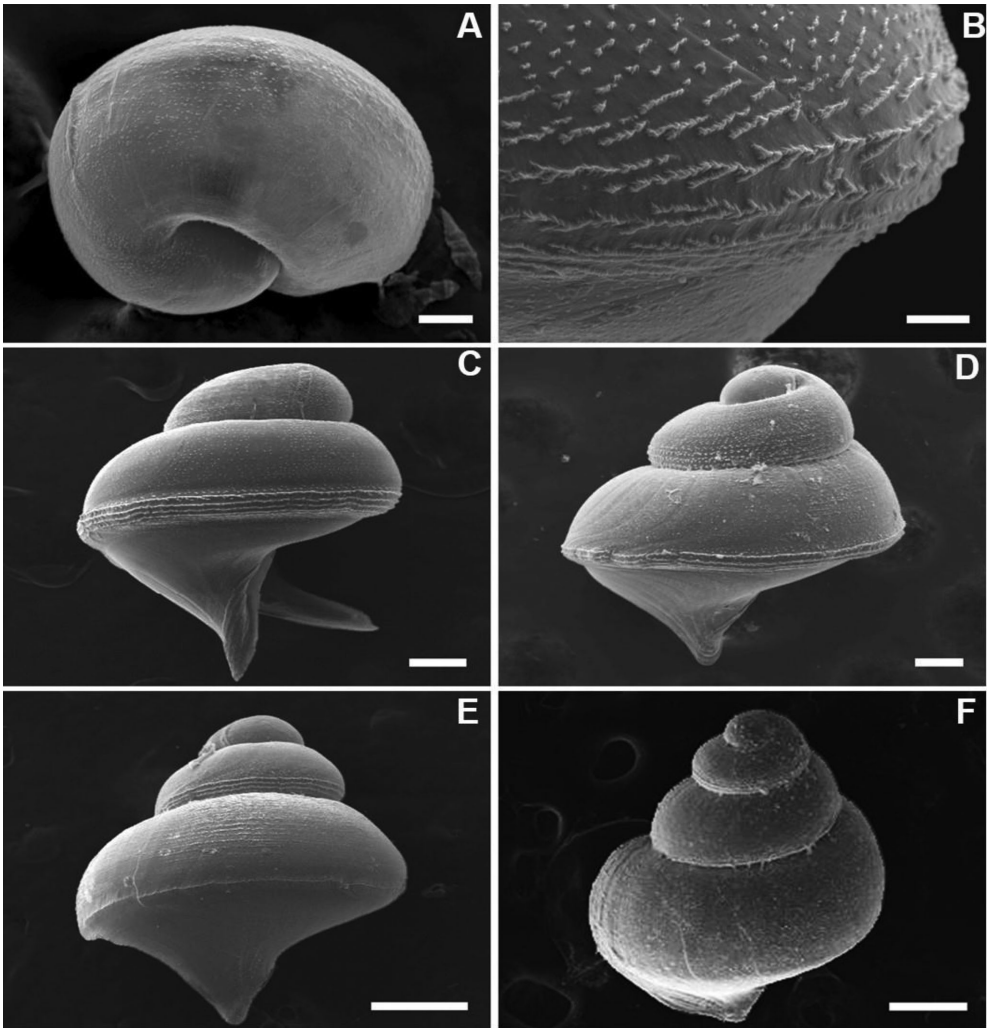

Figure 1. SEM views of larval shells from veligers to pediveligers and newly settled juveniles of *Strombus gigas*. **A.** Apex-up view of a newly hatched larva (1 d old) showing irregular raised sculpture; note that protoconch I is visible. **B.** Detail of 3-d-old larval shell showing ornamentation. **C.** A 9-d-old veliger larva showing first two shell whorls and a prominent beak. The raised sculpture is now well defined, with several close spiral ribs on the keeled periphery of the second whorl. **D.** Dorsal view of late veliger larva (15 d old). The shell has three whorls. The siphonal canal is flatter than that in early larvae. **E.** Dorsal view of 35-d-old late pediveliger showing three whorls. The shell ornamentation consists of close spiral lines on the third whorl. **F.** Dorsal view of 42-d-old, newly settled juvenile shell with four whorls. The characteristic striae are just visible. Scale bars: **A** = 50 μm; **B** = 25 μm; **C**, **D** = 100 μm; **E**, **F** = 250 μm.

was done using an M-max 50 mm^2 Oxford detector, monitored by an INCA system, in the point mode. Spectra were read after an acquisition time of 180 s. A total of 31 spherocrystals were analysed from nine different crypt cells. Cytoplasm from digestive cells and from the resin outside the tissue were analysed as negative controls.

RESULTS

Immediately after hatching, each larva had a protoconch that appeared uneven, and the shell width measured 365 ± 16 μm (Fig. 1A, B). In early veligers (2 d old), the digestive gland was visible through the transparent shell. Transverse semithin and thin sections of young larvae did not show cellular differentiation in the digestive gland (Fig. 2A–C). Tubules consisted of an epithelium with a single cell type, digestive cells, which were cuboidal and an average of 15 μm long by 6 μm wide. They formed a simple epithelium. Each cell had a basal nucleus surrounded by electron-dense droplets (probably vitelline granules) and some included granules of 4 ± 0.79 μm in diameter (n = 50) of heterogeneous content.

In late veliger stage (9 d old), each larva had a velum with four lobes and a siphonal length of 488 ± 38.63 μm (Fig. 1C). The stomach appeared as a straight tube connected to the digestive gland, which included a few distinguishable lobes. The digestive gland appeared darker in colour because the larvae had begun to ingest unicellular algae. Semithin and thin sections of digestive glands showed several tubules containing digestive cells (Fig. 2D–F).

In early pediveliger stage (17 d old), shell length was 562 ± 11.55 μm (Fig. 1D). Semithin transverse sections of the midgut revealed a stomach and digestive gland. The digestive gland was composed of digestive cells and crypt cells. The cytoplasm of crypt cells had small inclusions characterized by several concentric layers (Fig. 3A, B).

In late pediveligers (35 d old), the transformation from pelagic to benthic behaviour occurred as crawling behaviour began. Shell length was 1,190 ± 55.68 μm (Fig. 1E). The epithelium of the digestive tubule was composed of two cell types: digestive cells, which were characterized by numerous empty vesicles in the cytoplasm, and crypt cells that formed a simple epithelium (Fig. 3C, D). No vacuolated cells appeared in this epithelium. The digestive cells were tall columnar cells *c.* 40 μm long by 20 μm wide and were

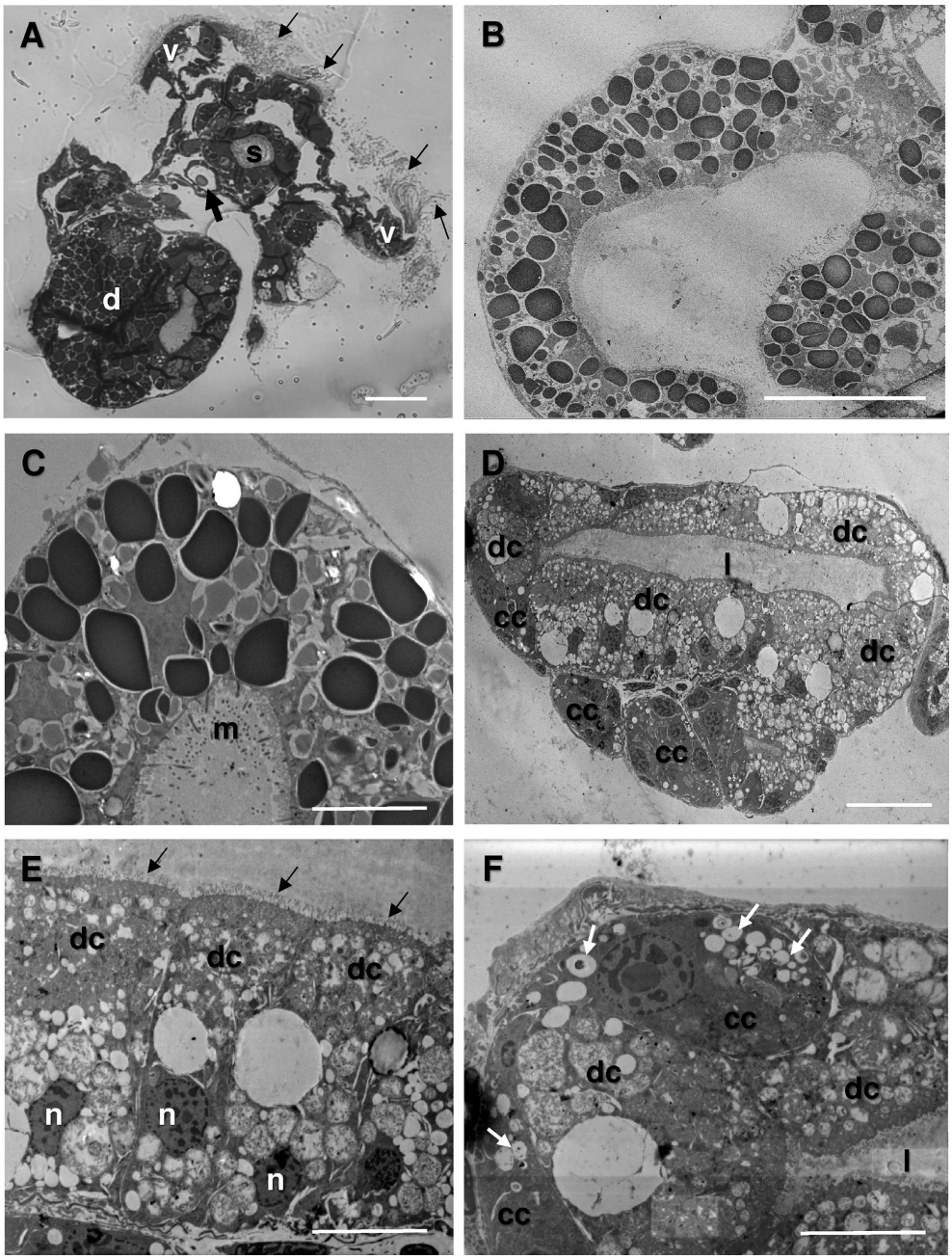

Figure 2. Early veliger larvae of *Strombus gigas*. **A–C.** Young veligers just after hatching (2 d after fertilization). **A.** Light micrograph of a semithin section of a newly hatched veliger showing the two-lobed velum with cilia extended (arrows). The digestive gland occupies the largest portion of the visceral mass; the thick arrow indicates a statocyst. **B.** STEM view of the digestive gland that appears to be composed of only one cell type with vitelline droplets appearing as electron-dense particles. **C.** Higher magnification view showing a few digestive cells characterized by long microvilli emitted from their apical pole projecting into the lumen. The electron-dense droplets are irregular in shape. **D–F.** STEM views of 9-d-old veligers. **D.** The digestive gland is composed of tubules that contain two cell types: digestive cells and the crypt cell. Each digestive cell has a basal nucleus and a cytoplasm characterized by numerous clear granules typical of this cell type. The cytoplasm of the crypt cell appears darker at this low magnification. **E.** Each digestive cell has a narrow columnar shape with a rounded apical pole covered by short microvilli (arrows). **F.** Crypt cells possess numerous small inclusions characterized by several concentric layers (white arrows) located in vacuoles. Abbreviations: cc, crypt cell; dc, digestive cell; dg, digestive gland; l, lumen of the tubule; n, nucleus; s, stomach; v, velum. Scale bars: **A** = 40 μm; **B** = 50 μm; **C** = 10 μm; **D** = 25 μm; **E**, **F** = 10 μm.

the most abundant cells in the digestive tubules. Each digestive cell had a basal nucleus surrounded by large granules (3–7 μm in diameter) with heterogeneous contents. The apical pole of the digestive cell contained smaller vesicles (0.5–2 μm in diameter) with a denser content (Fig. 3E, F). Crypt cells were characterized by their pyramidal shape and cytoplasm full of spherical inclusions identified as spherocrystals (Fig. 3D). Crypt cell size varied from 4 to 6 μm in length and from 2 to 4 μm in width. Each cell had a large nucleus (Fig. 3D) and a large rough endoplasmic reticulum. The spherocrystals were spherical inclusions about 1–2 μm in diameter and were composed of alternating electron-dense and electron-lucent concentric layers organized around a matrix core (Fig. 4). The EDX spectra obtained from various spherocrystals from two crypt cells (cc1 and cc2) indicate that such cytoplasmic granules contain

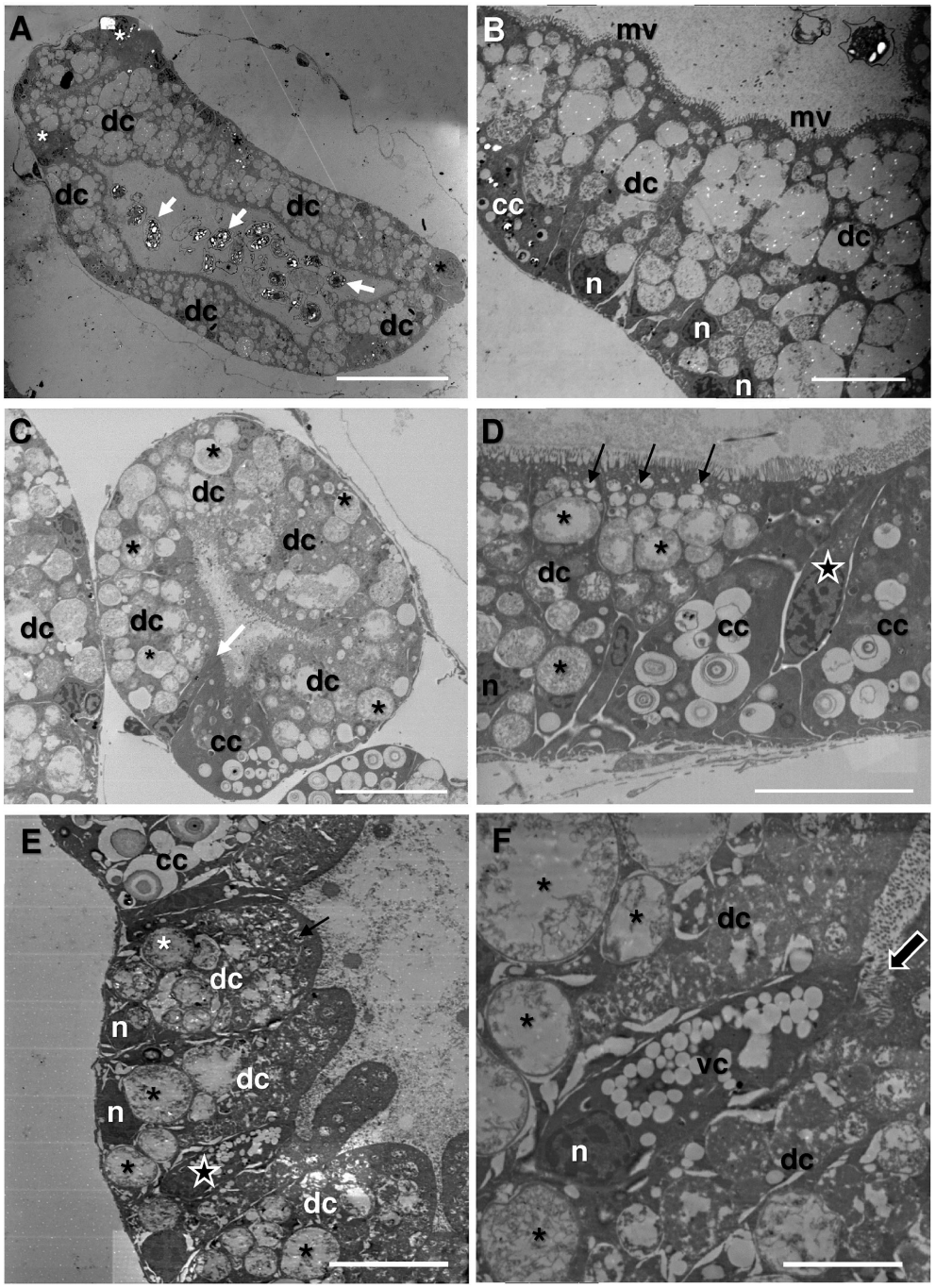

Figure 3. Ontogenesis of the digestive gland in pediveligers and newly settled juveniles of *Strombus gigas*. **A, B.** STEM views of early pediveligers 17 d after hatching. **A.** Low-magnification view of a tubule composed of two cell types, digestive cells (with their basal nuclei and cytoplasm filled with granules) and crypt cells containing spherocrystals. The lumen of the tubule contains numerous unicellular algae (arrows). **B.** At higher magnification, the organization of a digestive cell is visible, with an apical pole characterized by microvilli, a cytoplasm filled with granules and a basal nucleus. The crypt cells contain several spherocrystals with heterogeneous contents. **C, D.** STEM views of late pediveligers 35 d after hatching. **C.** Low-magnification view of three tubules containing digestive cells (with basal nuclei and cytoplasm filled with granules) and crypt cells containing spherocrystals. One narrow elongated cell (arrow) appears between the crypt and digestive cells; it may represent an immature stage of a third cell type (star). **D.** At higher magnification, the crypt cells are seen to contain several spherocrystals that appear to be more highly organized (i.e. with different internal layers) compared to those present in 15-d-old pediveligers. The microvilli at the apical poles of digestive and crypt cells are of different lengths. Digestive cells possess granules and small vesicles (arrows) close to their apical pole and a basal nucleus. **E, F.** STEM views of the digestive glands of young plantigrades 42 d after hatching. **E.** In plantigrades with shell length between 800 and 1,000 μm, the third cell type observed in the tubules of old pediveligers between the digestive cells now appears to be longer and more differentiated (star) and looks like the vacuolated cells described in adults. **F.** A vacuolated cell characterized by numerous small empty vacuoles that likely contained lipids lost during the dehydration and embedding processes. This vacuolated cell is located between two digestive cells and its apical pole is characterized by microvilli (arrow). No internal apicomplexan structure could be observed in its cytoplasm. Granules with heterogeneous contents filling the cytoplasm of the digestive cells are indicated by asterisks. Abbreviations: cc, crypt cell; dc, digestive cell; dg, digestive gland; l, lumen of the tubule; mv, microvilli; n, nucleus; nu, nucleus of the vacuolated cell; s, stomach; v, velum; vc, vacuolated cell. Scale bars: **A** = 50 μm; **B** = 10 μm; **C** = 20 μm; **D** = 10 μm; **E** = 20 μm; **F** = 5 μm.

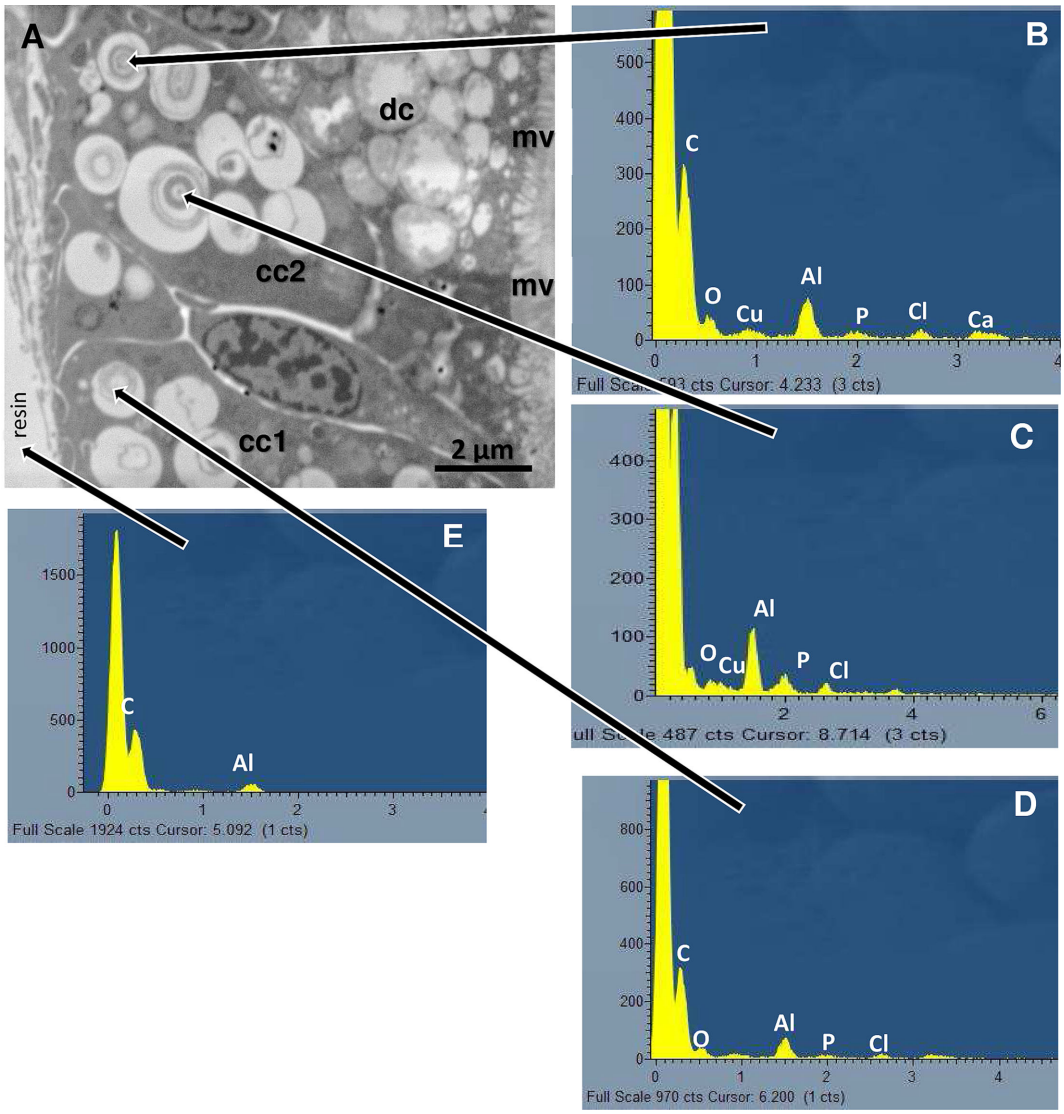

Figure 4. STEM view of crypt cells in a 35-d-old late pediveliger (**A**) and four associated EDX spectra (**B–E**) recorded at 20 kV. Three spherocrystals (black arrows) were analysed in two adjacent crypt cells (cc1 and cc2) close to a digestive cell that had a microvillus at its apical pole. Abbreviations: cc, crypt cell; dc, digestive cell; mv, microvillus.

Table 1. Elements detected by EDX analysis in spherocrystals of *Strombus gigas*.

	Elements (atomic %)					
	O	P	Cl	Ca	Cu	Z
Mean	91.7	1.2	2.18	0.15	4.49	0.278
Standard deviation	4.6	0.57	0.85	0.25	1.87	0.09
Maximum	96.37	1.47	2.60	0.28	5.88	0.29
Minimum	86.2	0.36	0.92	0	2.6	0.12

various minerals, such as chlorine, calcium and phosphorus, and trace metals, such as copper (Fig. 4, Table 1). The compositions of spherocrystals B and C (from the tubule of cc2) are quite different, suggesting that the composition of spherocrystals is heterogeneous within the same cell. The peak of Al detected in the four spectra was not representative of the spherocrystal composition as it was detected at a similar level from the resin outside the cell (Fig. 4E).

Aluminium was detected at similar levels from the spherocrystals or cytoplasm (data not shown) of digestive cells and from the resin (Fig. 4E), indicating that this metal comes from the specimen holders that support the grids under beam for EDX analysis. Thus, Al was not considered as belonging to the spherocrystals. The other elements were not detected from the cytoplasm of adjacent cells tested (i.e. digestive cells and vesicle cells) or from the resin, so they were considered as specific to spherocrystals. No coccidian endosymbionts were observed in any larvae or newly settled juveniles.

In newly settled juveniles (42 d old), no trace of velar lobes was observed and shell length was 1,323 ± 20.17 μm (Fig. 1F). A well-formed digestive gland was evident with the two characteristic cell types also found in adults: digestive and crypt cells (Fig. 3E, F). The crypt cells contained small concretions of less than 1 μm in diameter, with the same shape described in younger stages. These structures are typical of spherocrystalline inclusions. A third cell type, the vacuolated cell, appeared at this stage of development between the digestive cells (Fig. 3E, F). This cell type is characterized by a cytoplasm filled with small vacuoles that probably have a role in lipid storage.

DISCUSSION

Many studies of the digestive tract and digestive gland of microphagous and herbivorous gastropods have used species inhabiting the intertidal environment to investigate the influence of tidal rhythms on these structures (Merdsoy & Farley, 1973; Boghen & Farley, 1974; Nelson & Morton, 1979). Other studies have described the structure and function of the digestive gland in bivalves and microphagous gastropods (Morton, 1979; von Salvini-Plawen, 1988; Voltzow, 1994; Morse & Zardus, 1997; Rosenberg *et al.*, 1997; Strong, 2003). A review by Voltzow (1994) summarizes the research done on the digestive gland of Caenogastropoda, including several histological and ultrastructural descriptions of different feeding types (microphagous, herbivorous and 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 (Graham, 1939). Development of the planktotrophic veliger of the nudibranch *Doridella steinbergae* was studied by histological examination. In larvae competent to metamorphose, lipid deposits were observed in the left digestive gland and rudiments of the adult kidney (Bickell & Chia, 1979). 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, 1984). The gastropod digestive gland exhibits a more complex structure suggesting 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). A study of veligers from eight species of gastropods has revealed the structural uniformity of the alimentary tract up to the time of metamorphosis (Fretter & Montgomery, 1968).

The structure of the digestive gland of the herbivorous gastropod *Strombus gigas* has been studied because it harbours putative symbionts belonging to the Sporozoa group (Baqueiro *et al.*, 2007; Gros *et al.*, 2009; Volland *et al.*, 2010; Volland & Gros, 2012). Of the three cell types in the digestive gland's digestive tubules (digestive cells, vacuolated cells and crypt cells), the largest and most conspicuous are the vacuolated cells, characterized by large clear vacuoles that may have a function in lipid storage. These cells are interspersed with small, slender pyramidal cells called crypt cells. Spherocrystals have been observed in the crypt cells of various molluscs, such as bivalves (George *et al.*, 1984), gastropods (Howard *et al.*, 1981; Nott & Langston, 1989; Taïeb & Vicente, 1999; Gros *et al.*, 2009; Volland *et al.*, 2010, Volland & Gros, 2012) and cephalopods (Martoja & Marcaillou, 1993; Costa, Rodrigo & Costa, 2014). Digestive cells are mostly involved in intracellular digestion in *S. gigas* (Volland & Gros, 2012), vacuolated cells store lipids and host sporozoan symbionts (Gros *et al.*, 2009; Volland & Gros, 2012) and crypt cells are involved in detoxification, with numerous spherocrystals being used per cell (Volland *et al.*, 2018).

In the present study, larvae were found to have spherocrystals, which had spherical inclusions *c.* 1 μm in diameter and were composed of concentric layers. Spherocrystals are deposits of pyrophosphate salt that trap metal ions (Taylor, Simkiss & Graves, 1989). These structures were described as potential storage structures of minerals and trace metals involved in detoxification (Marigómez *et al.*, 2002; Delakorda *et al.*, 2008; Rodrigo & Costa, 2017). In strombids, the elements Ca, Cu, Fe, Mg, Mn, P and Zn have been previously detected in spherocrystals, suggesting that these structures could play a role in regulating minerals and essential trace metals in *S. gigas* (Volland *et al.*, 2012, 2018). Moreover, analyses of spherocrystals from faeces have shown that even after passing through the digestive system they contain a substantial proportion of trace metal content when compared with those in the digestive gland (Nott & Nicolaidou, 1996). Spherocrystals represent a detoxification pathway for trace metals in molluscs (Marigómez *et al.*, 2002). Both of the known detoxification pathways in strombids involve metallothioneins; the pathways are those involved in Cu and Zn detoxification (Volland *et al.*, 2018). EDX analyses have shown the spherocrystals to contain Ca, Cu, Cl, P and Zn, the same elements found in spherocrystals from adult conch (Volland *et al.*, 2012). Strombid larvae apparently employ detoxification via spherocrystals in the crypt cells.

In late pediveligers of *S. pugilis*, the digestive gland is reported to contain the three characteristic cell types described in adults (Enriquez-Diaz *et al.*, 2015). Vacuolated and digestive cells were the most abundant and were characterized by having numerous empty vesicles inside the cytoplasm. The third cell type was noticeably darker and probably corresponded to crypt cells. However, Enriquez-Diaz *et al.* (2015) based their findings only on semithin sections analysed with a light microscope (i.e. rather than an ultrastructural analysis from thin sections by STEM and/or TEM), and they unfortunately misidentified the vacuolated cells as digestive cells.

All adult specimens of *S. gigas* observed to date harbour symbiotic coccidians in the digestive gland (Aldana Aranda *et al.*, 2007, 2011). We suggest that Coccidia colonize individuals of *S. gigas* at some point after the metamorphic developmental stages. Cultivation of *S. gigas* larvae from fertilization to 1.5-mm-long juveniles in the present study gave us the opportunity to examine the process of coccidian symbiont transmission in the Strombidae. We observed around 45 thin sections and found no apicomplexan structures in the digestive cells of the larvae investigated (even after 42 d of development). The presence of vacuolated cells may, therefore, be a precondition for infestation by a free-living form of this coccidian intracellular symbiont. In conclusion, our findings suggest the hypothesis that Apicomplexa are acquired from the environment after metamorphosis in the benthonic stages of this species, when vacuolated cells are well developed in the digestive gland.

ACKNOWLEDGEMENTS

The research reported here was funded by a grant from the CONACyT (SEP 24210, Connectivity of Conch in the Caribbean, CFMC). We would like to thank the two anonymous reviewers who provided constructive comments and Associate Editor John Graham for his input in helping us to substantially improve the paper.

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