

RESEARCH NOTE

Occurrence of Sporozoa-like microorganisms in the digestive gland of various species of Strombidae

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The digestive gland of *Strombus (Eustrombus) gigas* Linnaeus, 1758 has been described by Gros, Frenkiel & Aldana Aranda (2009) as an assemblage of tubules and ducts that are connected to the stomach. Three cell types occur in the epithelium of the blind-ending tubules, according to histological and TEM observations: digestive cells, crypt cells and vacuolated cells. Several examples of parasitic relationships between marine gastropods and apicomplexan parasites have been described (Friedman *et al.*, 1995; Azevedo & Padovan, 2004). Baqueiro Cárdenas *et al.* (2007) reported the presence of intracellular microorganisms that they interpreted as belonging to various life cycle stages characteristic of Apicomplexa within some cells in the digestive gland of *S. gigas*. Heterospecific interactions between organisms living inside the cells of another one are not easy to characterize. The description of Sporozoa-like microorganisms occurring in the digestive gland of *S. gigas* raises several questions: How broad is the geographic distribution of the Sporozoa-like microorganism? Are other species of Strombidae also infected? The present study reports the occurrence of Sporozoa-like microorganisms from a structural analysis of the digestive gland of six species belonging to the family Strombidae found in distant geographic regions.

Individuals from four Caribbean species: *Strombus (Aliger) costatus* Gmelin, 1791 ($n = 50$); *S. (Aliger) gallus* Linnaeus, 1758 ($n = 1$); *S. (Eustrombus) gigas* ($n > 1,500$) and *S. pugilis* Linnaeus, 1758 ($n = 100$) and two species from the Pacific Ocean: *S. galeatus* Swainson, 1823 ($n = 1$) and *Lambis lambis* Linnaeus, 1758 ($n = 2$) were sampled. Specimens of *S. costatus*, *S. gallus*, *S. gigas* and *S. pugilis* were collected from Guadeloupe (French West Indies) and Yucatan (Mexico) in beds of the seagrass *Thalassia testudinum* or on coarse sand bottoms. Specimens of *L. lambis* were collected in 2006 from the Vanuatu Archipelago in the Pacific Ocean during the Santo 2006 expedition. The unique specimen of *S. galeatus* collected from the Pacific coast of Mexico was preserved in 10% formalin in seawater. The others samples were fixed in 5% glutaraldehyde in filtered seawater. Samples of the digestive gland were prepared for histological and ultrastructural analyses as previously described (Gros *et al.*, 2009).

The digestive glands of all six species were composed of an assemblage of digestive tubules and ducts. Three cell types were identified in the tubules: digestive cells, vacuolated cells and crypt cells (Fig. 1). The typical structures observed in the samples were two functional groups of digestive cells and vacuolar cells facing each other. Sporozoa-like microorganisms were detected in all sampled specimens, whatever the species (Fig. 1). When in host cells, these microorganisms were always found in vacuolated cells inside a parasitophorous vacuole. In a few cases, they were observed in digestive cells. We also

observed microorganisms in the lumina of tubules and ducts of all species, as well as in the faeces of *S. costatus*, *S. gigas* and *S. pugilis*. Faeces of *L. lambis*, *S. galeatus* and *S. gallus* could not be analysed. The microorganisms measured 10–60 µm long and 5–40 µm wide. Various life cycle stages were identified according to Perkins (1991), based on the presence or lack of a thick wall and/or an inner budding. The most frequent stage found was the thick-walled sporocyst, which was often damaged by sectioning. Typical bottle-shaped trophozoites characterized by an apical complex were observed. Gamont stages were characterized by thin walls. The digestive gland samples of *S. galeatus* were not fixed for TEM analysis. However, microorganisms similar to those found in the other Strombidae were observed in semi-thin sections of this species (Fig. 1F).

Sporozoa infections have already been described for several marine gastropods. *Pseudoklossia haliotis* was reported as a parasite of California *Haliotis* species (Friedman *et al.*, 1995). *Nematopsis gigas* was reported as a parasite of *Nerita ascensionis* (Azevedo & Padovan, 2004). Sporozoa is a very large clade of parasitic protists (including Apicomplexa), which comprises 4,000–7,000 species (Perkins, 1991; Cox, 1994). Several life cycle stages have been identified in the six strombid species, of which the most commonly described are: sporocyst, gamont and trophozoite. According to Friedman *et al.* (1995), the presence of various stages within the same host supports the hypothesis of a monoxenous life cycle. The presence of several life-cycle stages in digestive tubule cells of the six strombid species analysed in this study also supports this hypothesis. The occurrence of Sporozoa-like stages observed in all six species of Strombidae analysed in this paper is similar to a sporozoan infection. Although various life cycle stages of the presumed parasitic microorganisms were identified, no clinical evidence of disease was observed. Gros *et al.* (2009) have emphasized the opportunity of parasite dispersal through the host's faeces that allows parasite transmission to new hosts through the environment. The presence of the thick cellular walls of the Sporozoa-like microorganism in strombids analysed in this study supports the hypothesis that the microorganism might be dispersed through faeces, because these walls could provide protection against digestive enzymes. An environmental transmission mechanism involving a continuous release of the symbionts has been described in various marine invertebrates such as the squid *Euprymna scolopes*, which daily rejects 90% of its symbiotic bacteria which are then transmitted to other individuals (McFall-Ngai, 1998). Regardless of species, gender and gonadic maturity the digestive gland of all sampled specimens contained a large number of Sporozoa-like microorganisms (i.e. 100% prevalence). The fact that all the individuals of the six different species examined in this study were infected by

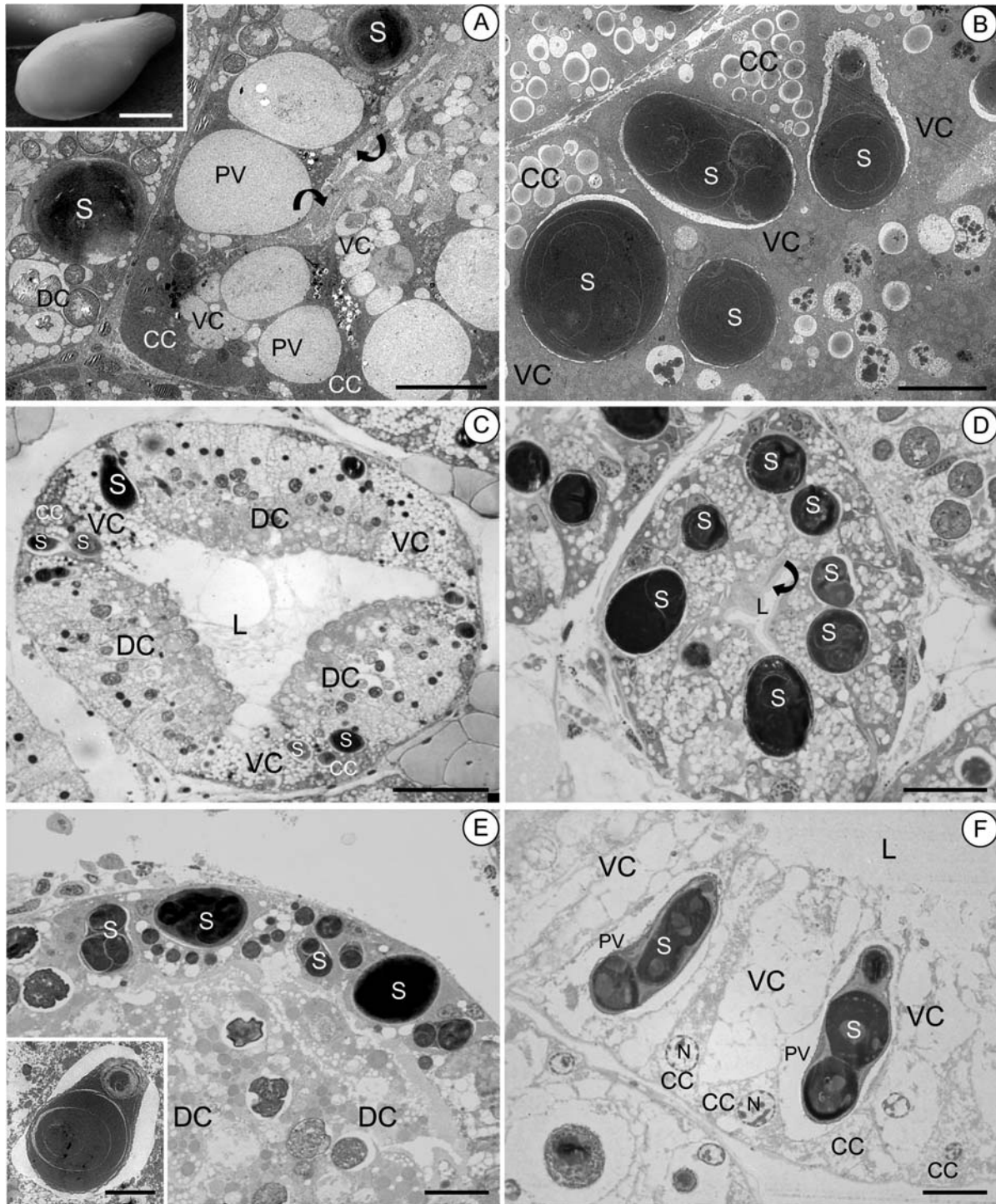


Figure 1. Micrographs of the digestive gland of strombid species showing the presence of Sporozoa-like organisms. **A.** *Strombus costatus*. TEM of the edge of a digestive tubule. The large electron-lucent vesicles correspond to Sporozoa-like organisms which appear weakly contrasted. Inset: SEM of a single Sporozoa-like organism obtained from a purified solution from the digestive gland of *S. costatus*. **B.** *Strombus gallus*. TEM of a digestive tubule showing various life stages typical of Sporozoa in vacuolated cells. **C.** *Strombus gigas*. Semi-thin section showing the three cell types that composed the digestive tubule and Sporozoa-like organisms in vacuolated cells. **D.** *Strombus pugilis*. Semi-thin section of a digestive tubule containing spherical Sporozoa-like organisms. **E.** *Lambis lambis*. Edge of a digestive tubule containing Sporozoa-like structures of various sizes and life stages. Inset: TEM of a single Sporozoa-like organism from *L. lambis*. **F.** *Strombus galeatus*. Semi-thin section showing two Sporozoa-like organisms in their parasitophorous vacuoles. Note the presence of triangular crypt cells interspaced between vacuolated cells. Abbreviations: CC, crypt cells; DC, digestive cells; L, lumen; N, nucleus; PV, parasitophorous vacuole; S, Sporozoa-like organism; VC, vacuolated cells. Curved arrows point to microvilli. Scale bars **A** = 20 μm , inset = 10 μm ; **B** = 10 μm ; **C** = 40 μm ; **D** = 20 μm ; **E** = 10 μm , inset = 5 μm ; **F** = 10 μm .

large numbers of these Sporozoa-like microorganisms contradicts one of the assumptions of a parasitic relationship (Combes, 1995). Moreover, Combes (2001) suggested that virulence should be defined by a decrease in the reproductive success of the host. Sporozoa-like microorganisms observed in two independent populations of *S. gigas* from Florida (Aldana Aranda *et al.*, 2009) and Guadeloupe (Frenkiel *et al.*, 2009), however, do not affect the reproductive cycle of this species. Similar low virulence was observed in *S. pugilis* by Baqueiro Cárdenas, Aldana Aranda & Martínez Olivares (2005). These observations suggest a relationship of very low virulence, so that these Sporozoa-like microorganisms could be commensal rather than parasitic in the Strombidae.

REFERENCES

- ALDANA ARANDA, D., GLAZER, R., DELGADO, G., BAQUEIRO CARDENAS, E. & MONTERO, J. 2009. Occurrence of Apicomplexa infecting queen conch, *Strombus gigas*, from off shore and near shore in Florida. *Proceedings of Gulf and Caribbean Fisheries Institute*, **61**: 491–497.
- AZEVEDO, C. & PADOVAN, I. 2004. *Nematopsis gigas* n. sp. (Apicomplexa), a parasite of *Nerita ascencionis* (Gastropoda, Neritidae) from Brazil. *Journal of Eukaryotic Microbiology*, **51**: 214–219.
- BAQUEIRO CÁRDENAS, E., ALDANA ARANDA, D. & MARTINEZ OLIVARES, G. 2005. Gonad development and reproductive pattern of the fighting conch *Strombus pugilis* Linné, 1758 (Gastropoda, Prosobranchia) from Campeche, Mexico. *Journal of Shellfish Research*, **24**: 1127–1133.
- BAQUEIRO CÁRDENAS, E., FRENKIEL, L., ZETINA ZARATE, A. & ALDANA ARANDA, D. 2007. Coccidian (Apicomplexa) parasite infecting *Strombus gigas* Linné, 1758 digestive gland. *Journal of Shellfish Research*, **26**: 319–321.
- COMBES, C. 1995. *Interractions durables*. Masson, Paris.
- COMBES, C. 2001. *L'art d'être parasite. Les associations du vivant*. Flammarion, Paris.
- COX, F.E.G. 1994. The evolutionary expansion of the Sporozoa. *International Journal for Parasitology*, **24**: 1301–1316.
- FRENKIEL, L., PRUVOST, L., ZETINA ZARATE, A., ENRIQUEZ DIAZ, M. & ALDANA ARANDA, D. 2009. Reproduction cycle of the queen conch *Strombus gigas* L. 1758. *Proceedings of Gulf and Caribbean Fisheries Institute*, **61**: 518–520.
- FRIEDMAN, C.S., GARDNER, G.R., HEDRICK, R.P., STEPHENSON, M., CAWTHORN, R.J. & UPTON, S.J. 1995. *Pseudoklossia haliotis* sp. n. (Apicomplexa) from the kidney of California abalone, *Haliotis* spp. (Mollusca). *Journal of Invertebrate Pathology*, **66**: 33–38.
- GROS, O., FRENKIEL, L. & ALDANA ARANDA, D. 2009. Structural analysis of the digestive gland of the queen conch *Strombus gigas* (Linnaeus, 1758) and its intracellular Apicomplexa parasites. *Journal of Molluscan Studies*, **75**: 59–68.
- McFALL-NGAI, M.J. 1998. Pioneering the squid-*Vibrio* model. *American Society for Microbiology News*, **64**: 639–645.
- PERKINS, F.O. 1991. 'Sporozoa': Apicomplexa, Microsporidia, Haplosporidia, Paramixia, Myxosporidia, and Actinosporidia. In: *Microscopic anatomy of invertebrates*. Vol. 1: Protozoa (F.W. Harrison & J.O. Corliss, eds), pp. 261–331. Wiley-Liss, New York.