Apicomplexan Parasite in the Digestive Gland of Various Species of the family Strombidae: *Strombus costatus*, *S. gigas*, and *S. pugilis*

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ABSTRACT

The present study aims to detect the presence of intracellular Apicomplexa in various species of the genus *Strombus. Strombus costatus, S. gigas,* and *S. pugilis*. Such presence of intracellular Protozoa associated with all individuals in every studied population and in all examined members of the same family raises diverse hypothesis. The parasitic interaction described in *S. gigas* extends to both other species of Strombidae studied. Such a relationship could occur in the whole Strombidae family. Further investigations will focus on strombid species from other geographic areas to confirm this hypothesis. Complementary investigations are also scheduled to ascertain the nature of relationship between Apicomplexa and the family Strombidae.

KEY WORDS: Apicomplexan parasite, Caribbean conchs, structure

Apicomplexa Parasito en la Glandula Digestiva de Varias Especies de la Familia Strombidae: *Strombus costatus*, *S. gigas*, y *S. pugilis*

El objetivo de este trabajo fue verificar la presencia de Apicomplexa en varias especies del género *Strombus* . La presencia de Apicomplexa intracelular en todos organismos de todas las poblaciones estudiadas de la misma familia plantean varias hipótesis. Este tipo de relación podria estar presente en toda la familia Strombidae. Se requiere de otras investigaciones sobre las especies de Strombus de otras localidades geográficas a fin de confirmar esta hipótesis. Estudios complementarios se están realizando para conocer la naturaleza del tipo de relación entre Apicomplexa y la familia Strombidae.

PALABRAS CLAVES: Apicomplexan parásito, caracol, estructura

INTRODUCTION

The five common species of the *Strombus* genus in the Caribbean are: *Strombus costatus* (Gmelin 1791)*; S. gallus* (Linnaeus 1758)*; S. gigas* (Linnaeus 1758), *S. pugilis* (Linnaeus 1758) and *S. raninus* (Gmelin 1791). Some of them are eaten (*S. costatus,* and *S. pugilis*) in various countries; however, *S. gigas* is the only species fished throughout the region and object of international trade. Previous observations of *S. gigas* digestive gland have shown several foreign structures in the population of San Andres Archipelago (Colombia). These structures have been defined as parasitic belonging to the Apicomplexa group (Aldana-Aranda *et al.* 2007). Apicomplexa is a group of Sporozoa containing about 5,000 species, all described as parasites. They are characterized by a complex life cycle composed of three phases: merogony (asexual reproduction), gametogony (sexual reproduction), and sporogony (asexual reproduction). Infecting units, formed by the second asexual reproduction are called trophozoits. This stage is characterized by an apical complex (Perkins 1991).

This study focuses on three Caribbean Strombidae: *S. costatus, S. gigas,* and *S. pugilis* .

MATERIALS AND METHODS

Individuals of *S. costatus, S. gigas,* and *S. pugilis* were collected from Guadeloupe (FWI) and Yucatan (Mexico) on *Thalassia testudinum* seagrass beds. Living materials were brought to the laboratory for analysis. The digestive glands were dissected and fixed in 5% glutaraldehyde in sea water for 24 hours at room temperature. Samples were then dehydrated through an ascending ethanol series and embedded either in paraplast wax for histology (5µm thick sections) or in epoxy resine for semi thin sections (0.5µm thick) according to standard methods. Histological sections were stained using a trichrome staining (hematoxylin, alcian blue, and acid fuchsine). Semi thin sections were stained using toluidine blue in borax.

RESULTS

For the three species, the digestive gland of every analyzed individual was infected by a large number of putative Apicomplexa organisms. In fact, the overall black colour of the digestive gland seems to be due to the presence of a huge number of parasites. Similar structures were observed in *S. costatus, S. gigas,* and *S. pugilis*. For each species, apicomplexan parasites occur most frequently in vacuolated cells (Figure 1.) but were also found in the lumen of tubules, in the collector ducts of the gland, and in the faeces. Sporocysts are spherical to ellipsoidal, they are always included in a parasitophorus vacuole (Figures 1-4). Their diameter is comprised between 10 and 60 µm with a thick, hard wall which is often destroyed by sectioning (Figures 2 and 4). Four different stages of Apicomplexa

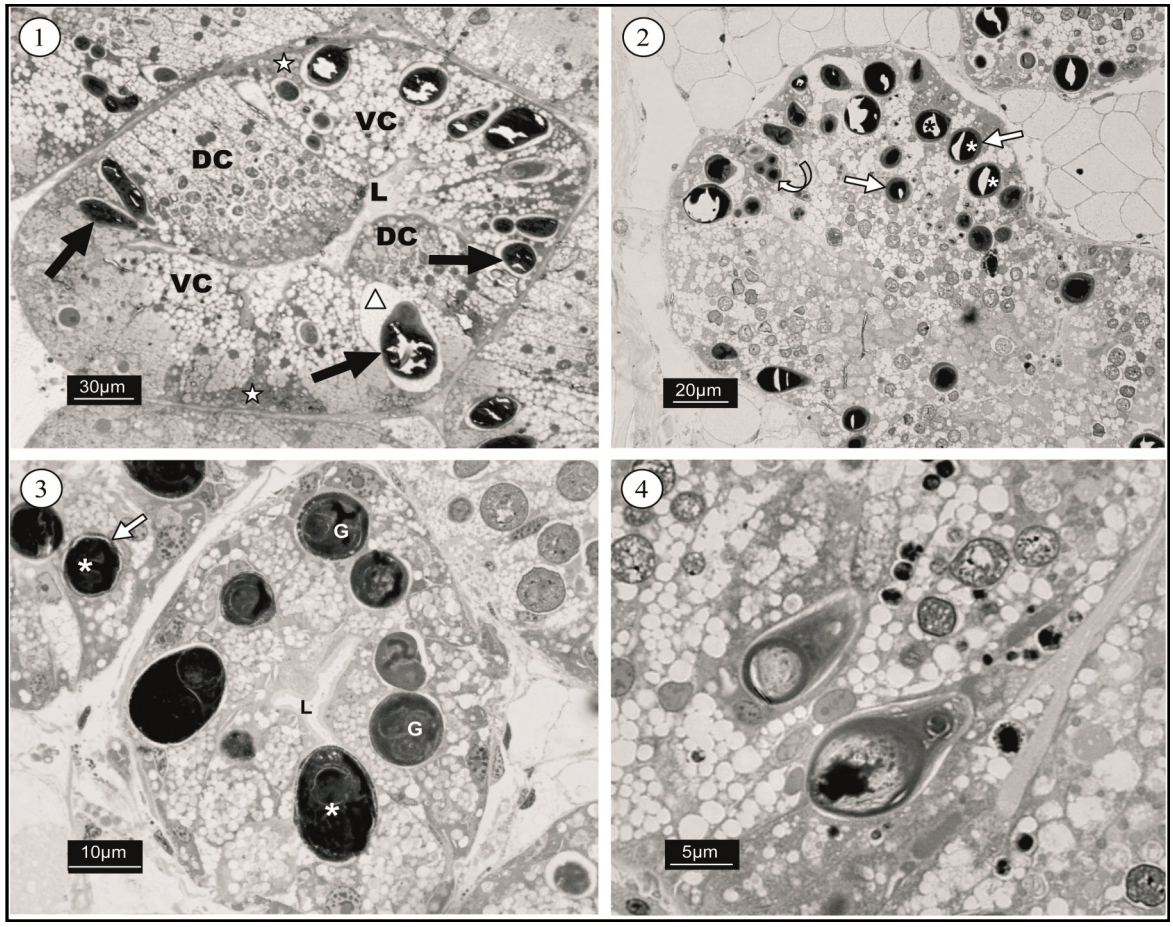
life cycle were usually observed in the digestive gland. The most frequent is the sporocyst stage with a thick wall described above (Figures 1 - 3). Some thin walled stages may be considered as gamont (Figure 3). Last but not least, typical bottle shaped organisms were frequently observed from the parasitic purified fraction but rarely on sections probably due to the section plan. This morphotype could be assimilated to the trophozoit stage, because of the presence of an apical complex characteristic of this stage and of the Apicomplexa group (Figure 4).

DISCUSSION

Apicomplexa have already been described in several molluscan species: *Nematopsis gigas* is a parasite of *Nerita ascencionis* (Azevedo and Padovan 2004); *Pseudoklossia haliotis* is a parasite of *Haliotis spp* (Friedman *et al.* 1995). Similar morphotypes as those observed here (thick walled sporocysts, thin walled gamonts, and trophozoits) have previously been described as different stages of Apicomplexa life cycle (Perkins 1991). Parasites are found in the faeces of the three conch species studied, that is potentially an excretion way for the parasite dissemination.

We emphasize that the parasitic interaction described in *S. gigas* extends to both other species of Strombidae studied. Such relationship could occur in the whole Strombidae family. Further investigations will focus on strombid species from other geographic areas to confirm this hypothesis.

For the three species investigated, we have observed the presence of the apicomplexan parasites in the digestive gland of all sampled individuals (n > 1000) from different Caribbean sites (Baqueiro *et al.* 2005, Baqueiro *et al.* 2007). This suggests to revaluate the assumption of parasitism and we propose to consider the possibility of a symbiosis relationship.



Figures 1-4. Light microscopy of semi-thin section of digestive gland of different Strombidae showing apicomplexan parasites in their parasitophorus vacuole in vacuolated cells. **Figure 1**. *S. gigas*'s digestive gland. **Figure 2**. *S. costatus*'s digestive glan. **Figure 3**. *S. pugilis*'s digestive gland. **Figure 4**. Detail of a trophozoit observed in digestive cells of *S. costatus*'s digestive gland. Black arrows point on apicomplexan parasites. White arrows point on the thick wall of the sporocysts. Curved arrow points the thin wall of the gamont. Asterisk: sporocyst; stars: cryptic cells; triangle: parasitophorus vacuole; DC: digestive cells; G: gamont; L: lumen of the tubule; VC: vacuolated cells.

Phylogenetic analysis is in progress in our lab in order to confirm that these endobiont-like structures belong to the Apicomplexa group. Moreover, physiological studies should be performed to clearly determine the nature of the interaction between conchs and their endobionts.

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