Detection of Apicomplexa like Parasites in Two Species Belonging to the Family Strombidae: *Strombus gallus*, Linnaeus, 1758 and *S. raninus*, Gmelin, 1791.

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ABSTRACT

The present study aims to detect the presence of intracellular Apicomplexa in two species of the genus *Strombus*: *Strombus gallus* and *S. raninus*. This parasitic interaction was initially described in *S. gigas* and extends to both other species of Strombidae studied. Such relationships 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

Puesta en Evidencia de Présencia del Parasito Apicomplexes en dos Especies de la Famillia des Strombidae: *Strombus galas*, Linnaeus, 1758 y *S. raninus*, Gmelin, 1791

El estudio de la glándula digestiva de *S. gigas* puso en evidencia la presencia de una infección importante y generalizada. Una cantidad importante de estructuras de tipo Apicomplexa fueron detectadas en las células vacuolares de los túbulos digestivos de todos los individuos muestreados. En este estudio, se analizó la glándula digestiva de dos otros *Strombidae* del Caraïbe: *S. gallus* y *S. raninus*. Las glándulas digestivas de los organismos fueron fijadas y el análisis estructural fue realizado con técnicas de histología y de microscopia electrónica y a transmisión. Para las dos especies, los tùbulos digestivos se componen de tres tipos celulares: células digestivas, células vacuolares y células crípticas. Este estudio puso en evidencia la presencia de estructures semejantes a los Apicomplexa, parecidos a lo observado en *S. gigas*, en las células vacuolares de *S. gallus* y *S. raninus*. Para las dos especies, los estadios del desarrollo característico del ciclo de vida de los Apicomplexa fue observado: El estadio trofozoïto (caracterizado por su compleja estructura apical), el estadio esporozoïto (con sus esporas internas) y el estadio gamont (caracterizado por una pared fina). Los parásitos de *S. gallus* midieron de 10 à 20 µm de diámetro y los de *S. raninus* en promedio fueron dos veces más grandes. Así, los dos *Strombidae* analizados tuvieron la presencia de parásitos que nos recuerdan a los Apicomplexa. Sin embargo, ningún síntoma de mala salud como incipiente desarrollo gonádico o desorganización de los túbulos digestivos fueron observados. Este estudio puso en evidencia que la infección descrita inicialmente en *S. gigas*, se presenta también en otras dos especies del mismo genero *Strombus*.

PALABRAS CLAVES: Apicomplexan parásito, caracol, estructura

Mise en Évidence de la Présence de Parsites Apicomplexes chez deux Éspecies de la Famille des Strombidae: *Strombus gallus*, Linnaeus, 1758 et *S. raninas*, Gmelin, 1791

L'étude de la glande digestive de *S. gigas* a révélée la présence d'une infection importante et généralisée. Une quantité importante de structures rappelant les Apicomplexes a été détectée dans les cellules vacuolaires des tubules digestifs de tout les individus échantillonnés. Dans cette étude, nous avons analysé la glande digestive de deux autres *Strombidae* de la Caraïbe : *S. gallus* et *S. raninus*. Les glandes digestives des individus ont été fixées et des analyses structurelles ont été réalisées en faisant appel aux techniques d'histologie et de microscopie électronique à transmission. Pour les deux espèces, les tubules digestifs se composent de trois types cellulaires : les cellules digestives, les cellules vacuolaire et les cellules cryptiques. Notre étude a mis en évidence la présence de structures rappelant des Apicomplexes, semblables à celles observées chez *S. gigas*, dans les cellules vacuolaires de *S. gallus* et *S. raninus*. Pour les deux espèces, les stades de développement caractéristiques du cycle vital des Apicomplexes ont été observés : le stade trophozoïte (caractérisé par son complexe apical), le stade sporozoïte (avec ses bourgeonnements internes) et le stade gamonte (caracterisé par une paroi fine). Les parasites de *S. gallus* font de 10 à 20 µm de diamètre et ceux de *S. raninus* sont en moyenne deux fois plus gros. Ainsi, les deux *Strombidae* analysés ont révélés la présence de parasites rappelant les Apicomplexes. Cependant, aucun signe de mauvaise santé tel qu'un retard de développement de la gonade ou une désorganisation des tubules digestifs n'a été observé. Cette étude a donc mis en évidence que l'infection décrite initialement chez *S. gigas*, s'étend à deux autres espèces du même genre.

MOTS CLÉS: Parasite Apicomplexe, lambis, structure

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 consumed (*S. costatus*, *S. gigas*, 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 (Baqueiro-Cárdenas *et al.* 2005, 2007, Gros *et al.* 2009). 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 two Caribbean Strombidae: *S. gallus* and *S. raninus*.

MATERIALS AND METHODS

Individuals of *S. gallus* and *S. raninus* were collected from Guadeloupe (FWI) on *Thalassia testudinum* seagrass beds. Living materials were brought to the laboratory for analysis. The digestive glands were dissected and fixed in 5% glutaraldehyde and 2% paraformaldehyde in sea water overnight at 4°C. 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. Thin sections were obtained from samples embedded in epoxy resin and contrasted with uranyl acetate and lead citrate before observation in a Leo 912 transmission electronic microscope (TEM).

RESULTS

The digestive gland of both species was composed by an assemblage of digestive tubules and ducts. Digestive tubules epithelium was composed by three cell types: long and columnar digestive cells, vacuolated cells and smaller triangular cells identified as crypt cells (Figures 1, 2).

Digestive gland of *S. gallus* and *S. raninus* was infected by a large number of putative Apicomplexa-like organisms. For each species, Apicomplexa-like parasites occur most frequently in vacuolated cells (Figres 1 - 3, 6) but were also found in the lumen of tubules and in the collector ducts of the gland (not shown). Sporocysts are spherical to ellipsoidal, they are always included in a parasitophorus vacuole. Their diameter is comprised between 10 and 30 µm (21 µm on average) in *S. gallus* and between 20 and 50 µm (36 µm on average) in *S. raninus* with a thick, hard wall which is often damaged by sectioning (Figres 5, 8). Three 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 5, 8). Some thin walled stages may be considered as gamont. Last but not least, typical bottle shaped organisms were observed. This morphotype could be assimilated to the trophozoite stage, because of the presence of an apical complex characteristic of this stage and of the Apicomplexa group (Figures 4, 7).

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).

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. Previous studies have highlight the presence of the Apicomplexa-like parasites in the digestive gland of all *S. gigas* sampled individuals (n > 1000 for *S. gigas*) from different Caribbean sites (Baqueiro-Cárdenas *et al.* 2005, 2007, Gros *et al.* 2009). We add two other species at the list of infected Strombidae: *S. gallus* and *S. raninus.* This suggests to re-evaluate the assumption of parasitism, and we propose to consider the possibility of a non-virulent relationship like commensalisms or even mutualism.

Phylogenetic analysis is in progress in our laboratory in order to confirm that these Apicomplexa-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 Apicomplexa-like.

LITERATURE CITED

Azevedo, C. and I. Padovan. 2004. *Nematopsis gigas* sp. (Apicomplexa), a parasite of *Nerita ascencionis* (Gastropoda, Neritidae) from Brazil. *Journal of Eukaryotic Microbiology* **51** (2):214-219

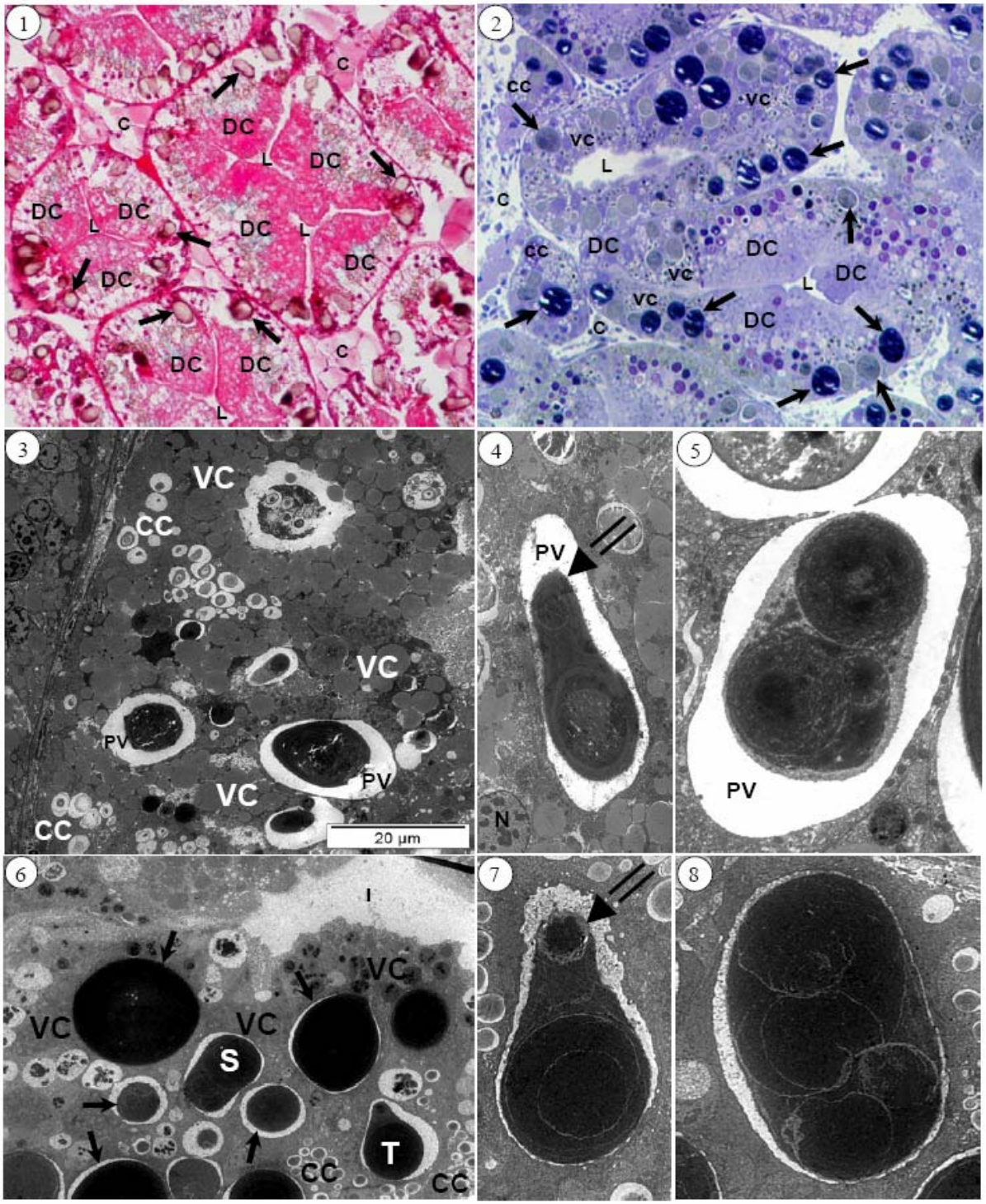
Baqueiro-Cárdenas, E., L. Frenkiel, and D. Aldana Aranda. 2005**.** One more threat for the Queen conch *Strombus gigas*? Coccidian (Apicomplexa) infection of *S. gigas* digestive gland: preliminary results. *Proceedings of the Gulf Caribbean Fisheries Institute* **58**:421-426

Baqueiro-Cárdenas, E., L. Frenkiel, A. Zetina Zarate, and D. Aldana Aranda. 2007. Coccidian (Apicomplexa) parasite infecting *Strombus gigas* linné, 1758 digestive gland. *Journal of Shellfish Research* **26**(2):319-321

Friedman, C.S., G.R. Gardner, R.P. Hedrick, M. Stephenson, R.J. Cawthorn, and S.J. Upton. 1995. *Pseudoklossia haliotis* sp. n. (Apicomplexa) from the kidney of california abalone, *Haliotis* spp. (Mollusca). *Journal of Invertebrate Pathology* **66**(1):33-38

Gros, O., Frenkiel, L., and D. Aldana Aranda. [2009]. Structural analysis of the digestive gland of the Queen conch *Strombus gigas* (Linnaeus, 1758) and its intracellular Apicomplexa parasites. *Journal of Molluscan Studies* [In press] 75(1).

Perkins, F.O. 1991. "Sporozoa": Apicomplexa, Microsporidia, Haplosporidia, Paramixea, Myxosporidia, and Actinosporidia. Pages 261-331 in: F.W. Harrison (Ed.) *Microscopic Anatomy of Invertebrates. Volume 1 Protozoa.* FW Harrison and Corliss New York, Chichester, Brisbane, Toronto, Singapore



Figures 1-8 . Observations of *S. gallus* and *S. raninus* digestive gland.
1-2: Histological observation of *S. raninus* (**1**) and semi-thin section of *S. gallus* (**2**) digestive gland showing digestive tubules mostly composed by digestive cells (**DC**). Parasites (**arrows**) are grouped in vacuolated cells (**VC**) between digestives cells. They are often damaged by sectioning. **CC:** crypt cells. **L:** lumen of the tubule. **3:** TEM views of *S. raninus* digestive gland showing Apicomplexa-like parasites in vacuolated cells. Note the presence of two crypt cells interspersed with vacuolated cells. **4-5:** TEM view of *S. raninus* digestive gland showing a trophozoite (**4**) characterized by the presence of an apical complex (**double arrow**) and a sporocyst (**5**) with its inner budding. **6:** TEM view of *S. gallus* digestive gland. Note the presence of different life stages (trophozoite: **T** and sporocyst: **S**) in vacuolated cells. **7-8:** TEM detail of *S. gallus* trophozoite (**7**) and sporocyst (**8**).