# One More Threat for the Queen Conch *Strombus gigas*? Coccidian (Apicomplexa) Infection of *S. gigas* Digestive Gland: Preliminary Results

ERICK BAQUEIRO CÁRDENAS[1], LILIANE FRENKIEL[2], and DALILA ALDANA ARANDA[3]
*[1]CICATA IPN*
*Altamira, Mexico,*
*2Archipel des Sciences*
*Guadeloup, French West Indies, France*
*3 CINVESTAV IPN Unidad Mérida*
*Laboratorio de Biología y Cultivo de Moluscos,*
*Km 6 antigua carretera a Progreso*
*C.P. 97310. Mérida, Yucatán, México*

**ABSTRACT**

An intense and generalized sporozoan infection was detected during reproductive studies of *Strombus gigas* populations from various localities of the Caribbean. The parasite, apparently a Coccidian, was found in the digestive gland (DG) of every sampled organism throughout the year, infecting from 70% to 100% of the DG alveoli, with a frequent total invasion of every alveolar cell. This infection is apparently responsible for the low intensity of maturity and spawning stages registered at San Andres Archipelago, Colombia, and Alacranes reef, Mexico. These findings induce several questions: Given the generalized infection of the two populations at so distant sites, if the parasite is the same, are *S. gigas* populations more connected than it is supposed? What are the environmental factors inducing such an intense and generalized infection? What are the life cycle, vectors, and intermediate hosts of this parasite? Is it a threat to human health? What is the impact on recruitment of the consequently attenuated reproduction?

Now more than ever, concerted actions have to be taken to solve these problems in order to obtain additional information on the putative parasites, prevent its expansion which could result in a collapse of conch populations, as well as a possible human health problem.

KEY WORDS: *Strombus gigas*, Coccidiasis, sporozoan, conch, disease, reproduction

## Una aMenaza más para el Caracol Rosa *Strombus gigas*? Coccidiasis (Apicomplexa) en la Glándula Digestiva de *S. gigas:* Resultados Preliminares

Una infección intensa y generalizada por esporozoarios fue detectada durante estudios de ciclos reproductores de *Strombus gigas* en San Andrès (Columbia) y arrecife Alacranes (México). El parásito, aparentemente un Coccidio se encontró en la glándula digestiva de los organismos analizados con un grado de infección del 70% al 100%. Aparentemente, esta infección es responsable de la baja intensidad en la madurez y desove registrados en las localidades del archipiélago de San Andrés, Colombia. Estos hallazgos inducen varias preguntas: Dada la intensidad y amplitud de la infección en tres poblaciones tan distantes, si se trata de un mismo organismo, ¿Se encuentran las poblaciones de *S. gigas* mas interconectadas que lo que se presuponía? Cuales son los factores ambientales que inducen una infección tan intensa y generalizada? ¿Cual es el ciclo de vida del parásito, cuales son sus vectores u hospederos intermediarios? ¿Representa este parásito una amenaza para la salud humana? ¿Cual es el impacto en el reclutamiento de esta reproducción atenuada?

Ahora más que nunca se requiere de acciones concertadas para resolver el problema con el propósito de prevenir la expansión del parásito, un colapso en las poblaciones y un probable problema de salud humana.

PALABRAS CLAVES: *Strombus gigas*, coccidiasis, esporozoarios, caracol, ciclos reproductores

## INTRODUCTION

The digestive gland of mollusks has been described for bivalves (Andrew 1959, Galtsoff 1964), a few gastropods (Andrew 1959) and cephalopods (Andrew 1959, Semmens 1998). They all coincide on the basic structure of digestive gland, composed of two types of columnar cells with basal nuclei, for which several functions have been identified. Saint-Hilaire and Young (in Galtsoff 1964) proved the ingestion of small food particles by the digestive cells. A role in the fixation of calcium phosphate and calcium carbonate for shell formation has been proposed by Jones and Saleuddin (1978), and Fournie (1979) among others. The controversial work of Semmens (1998) proposes an excreting role for nonstructural lipids in cephalopods. Andrew (1959) mentions that "the presence of solid bodies within the liver cells is common in mollusks". Such inclusions are considered as residual bodies of lysosomal digestion.

On the other hand, Coccidian, Apicomplexa are frequent parasites of Mollusks. Unfortunately, most work has been done on oysters, and other bivalves. They have been confirmed in abalone (Lester and Davis 1981) and shipworms (Hillman *et al*. 1982), as well as in Cephalopods (Gonzalez *et al*. 2003). The Apicomplexa are a monophyletic group composed almost entirely of parasitic species. Apicomplexa, along with ciliates and dinoflagellates, form a higher order group known as Alveolata. Formerly the Apicomplexa were part of a group called sporozoa and this name is still sometimes used. Their name is given by reference to a characteristic apical group of organelles. Apicomplexa have a complex life cycle, characterized by three distinct processes: sporogony, merogony and gametogony. The various stages may involve various hosts, but frequently they can perform their whole life cycle within the same or various cells of a single host. The coccidia are characterized by a thick walled oocyst stage that is typically excreted with the feces.

## MATERIAL AND METHODS

### Study Area

*San Andres, Colombia* — the Archipelago of San Andres, Colombia; between 12º and 16º N and 78º and 82º W covers an area of 300,000 km$^2$ of territorial sea and exclusive economic zone of Colombia, in the southern Caribbean sea.

*Alacranes Reef, Mexico* — is an oval-shaped bank reef formation, covering an area of 293 km$^2$ with a maximum length and width of 26.5 km and 14.8 km, respectively. It is located on the north coast of Yucatan, 80 miles off the coast, between 22$^o$ 21' and 22$^o$ 35' N and between 89$^o$ 38' and 89$^o$ 49' W.

### Collection and Histological Procedure

Thirty specimens (larger and equal to 20 cm of total shell length) were collected monthly, during one year, by free diving around San Andrès islands for the Colombian samples. For Alacranes, 30 specimens were collected monthly by free diving. All organisms had a well formed shell lip, independently of their size.

Specimens were dissected on site. Extraction of soft parts was achieved by boring a hole on the third turn of the spire, cutting off the columellar muscle. A one cubic centimeter from the middle region of the visceral mass which included a sample of the gonad and digestive gland was dissected and pre-fixed for 12 to 15 days in 10% buffered formalin prepared in filtered seawater with sodium borate. For transportation, samples were preserved in 70% alcohol with 0.1% glycerin (Bondad-Reantaso *et al*. 2001). They were post-fixed in the laboratory, for seven days in alcoholic Bouin´s fixative; and processed by routine histological techniques. Harris's Hematoxylin and Eosin method was used for staining (Luna 1968).

## RESULTS

Large inclusions in the digestive diverticular cells have been interpreted as an intense and generalized Sporozoan infection. The parasite, apparently a Coccidian, Apicomplexa was found in the digestive gland (DG) of every organism analyzed throughout the year from the archipelago of San Andres, Colombia and Alacranes reef, Mexico. Figures 1 through 5 present different levels of cysts and spores infestation. A low intensity infestation is presented in figure1 where a few digestive diverticula are free from parasites, and only some cells are infected in others. It points out the presence of typical bottle shaped and spherical cysts in ducts. Figure 2 presents bottle shaped and spherical cysts, pointing out the isolating reaction of the cell around a foreign body, and a microspore forming cyst. A detailed view of spherical cysts and isolating reaction is presented in Figure 3. Figure 4 presents a bottle-shaped cyst, macro and micro cysts, pointing out the hyaline wall of macrospores, and spores. Clear circles are the location of displaced cysts. Detailed views of spore-forming cysts and just released spores are presented in Figure 5. The presence of cysts in the digestive gland is constant through the year at both localities, with a striking similarity in intensity. Table 1 presents the main characteristics observed. Incidence of cysts is lower from January to May, with a minimum during March and April and maximum during October and November. From August to December, two types of spores could be detected. Microspores are about the diameter of the columnar cell nucleus; they are very dense and stain in deep red with the Hematoxyline – Eosin technique used. Macrospores are about twice as large in diameter; they present a granular cytoplasm and frequently "U" shape granular structure can be seen (Figures 6 - 7). The diagrammatic representation of various cyst shapes detected is presented in Figure 8. Bottle shape cysts frequently have a complex structure associated to the ring on what is assumed to be the apical zone. This end of cyst (a, b and d) was usually the only portion of the cyst in contact with the cell cytoplasm. Round macro and micro cysts were so dense that any structures could be seldom identified inside, except for spore forming cysts in which a various number of hyaline structures could be seen (f). Structures h and i had the same size and shape as a spore-forming cyst, but two rings were clearly identified. However, no structures could be detected inside.

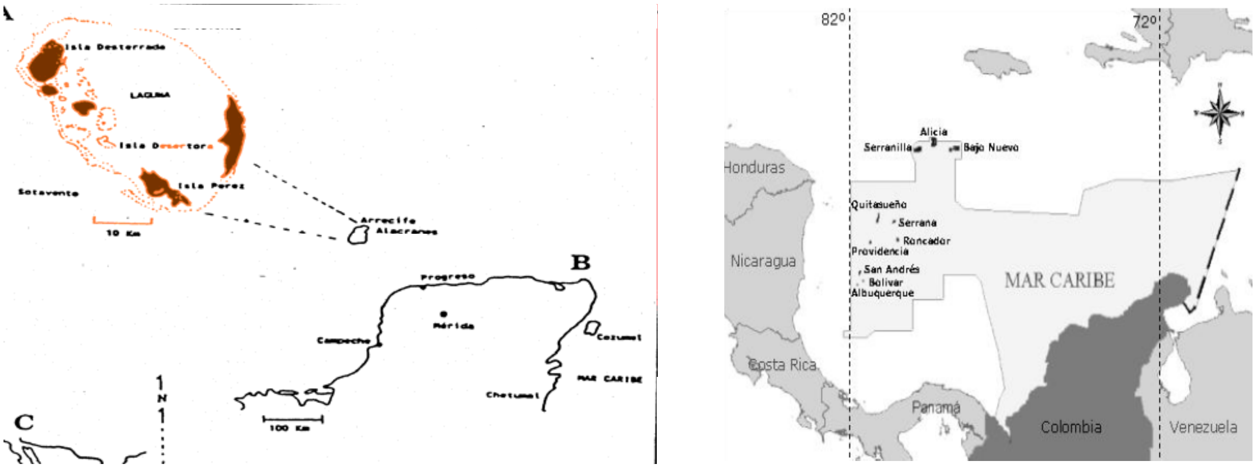


**Figure 1.** Sampling sites, a) Alacranes reef, Mexico; b) San Andres archipelago, Colombia.

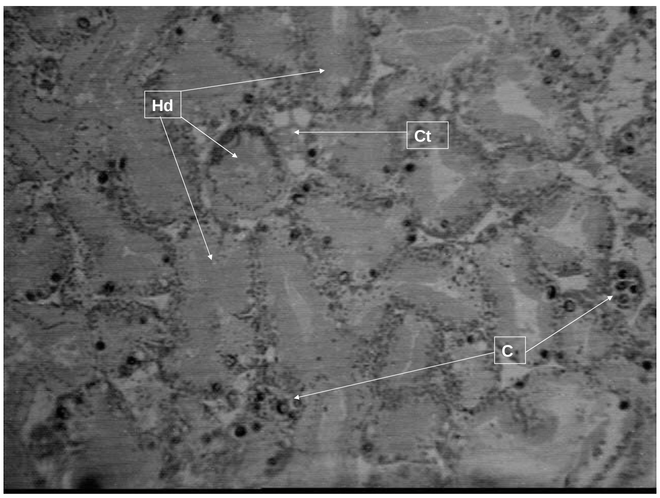


**Figure 2.** General vew of a partialy infected digestive gland of *S. gigas*. C, cysts of sporozoa; Hd, healthy digestive diverticula; Ct, connective tissue (10 x).

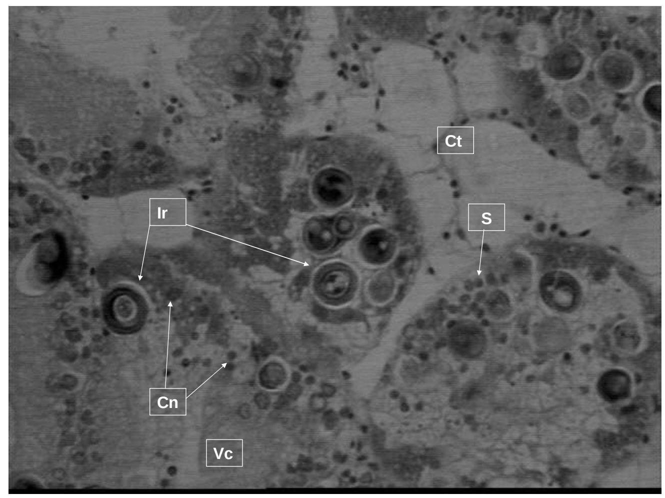


**Figure 4.** Detail of digestive diverticula showing different development stage of the parasite. Cn, cell nucleus; Ct, connective tissue; Ir, isolating reaction; S, spores. Vc,

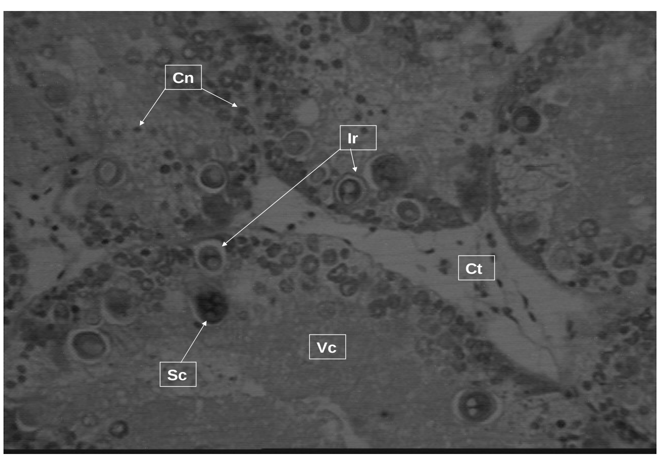


**Figure 3.** Detail of digestive diverticula, where cyst can be seen with a clear alo showing isolating reaction from the cell.; Cn, Cell nucleus; Ct, Connective tissue; Ir, Isolating reaction; Sc. Spore forming cyst, c, Vacuolar cytoplasm (40 x).

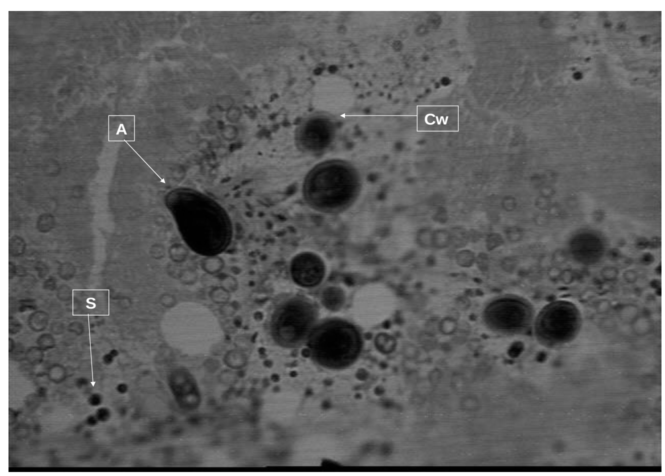


**Figure 5.** Detail of cysts where the typical Apicomplexa shape can be seen, with apical region. A, Apicomplexa like cyst; Cw, cyst wall; S, spores. (40 x).

**Table 1.** Principal characteristics observed on the digestive gland of *S. gigas* from San Andrès, Colombia and Alacranes , Mexico. A, Abundant(>50); F, few (<50); S, scarce; B, bottle-like cyst; BC, big spherical cyst; SC, small spherical cyst; BS, big spore; SS, small spore.

| | **Localities** | | |
|---|---|---|---|
| **Months** | **Alacranes** | **San Andrés** | |
| January | F, B, BC, SC | | Cysts in different segmentation stages.<br>Cysts in lumen of digestive gland ducts and digestive track. |
| February | F, B, BC, SC | | Few cysts in segmentation.<br>Cysts in lumen of digestive gland ducts and digestive track |
| March | F, B, BC, SC | F, B, BC, SC | Cysts in different segmentation stages, more abundant than in January and February<br>Cysts in lumen of digestive gland ducts and digestive track |
| April | A, B, BC, SC | A, B, BC, SC | Few cysts in segmentation.<br>Over 70% of healthy hepatic tissue Cysts in lumen of digestive gland ducts and digestive track |
| May | F, B, BC, SC | F, B, BC, SC | Few cysts in segmentation.<br>Over 70% of healthy digestive tissue Cysts in lumen of digestive gland ducts and digestive track |
| June | | A, B, BC, SC | Numerous cysts in lumen of digestive gland ducts and digestive track |
| July | | | |
| August | A, B, BC, SC, BS, | A, B, BC, SC | Cysts in different segmentation stages |
| September | A, B, BC, SC | A, B, BC, SC | Few healthy digestive gland cells, all diverticula with cysts |
| October | F, BS, Ss | A, B, BC, SC | Few healthy digestive gland cells, all diverticula with cysts |
| November | A, B, BS, SS | A, B, BC, SC, BS, SS | Few healthy digestive gland cells, all diverticula with cysts |
| December | A, B, BC, SC, BS, SS | A, B, BC, SC, BS, SS | Few healthy digestive gland cells, all diverticula with cysts |

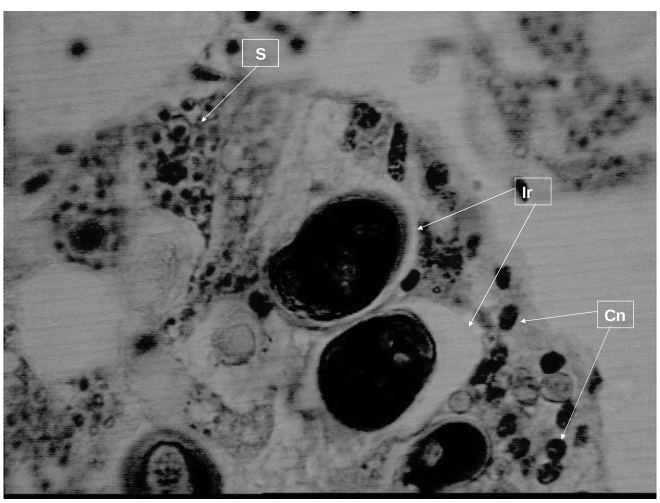


**Figure 6.** Digital image of cysts and spores. Cn, cell nucleus; Ir, Isolating reaction; S, spores. (100 x)

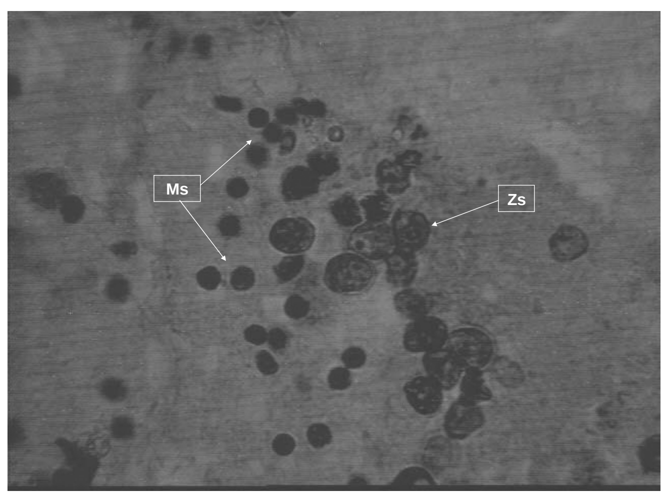


**Figure 7.** Digital image of digestive diverticula of *Strombus gigas* with two types of spores. Ms, microspores; Zs, zoospores or macrospores (10 x)

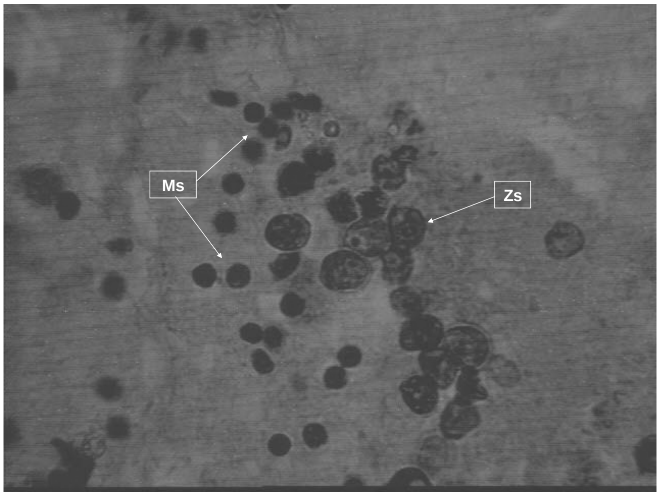


**Figure 8.** Detail view of spores, Ms, microspores; Zs, zoospores o macrospores

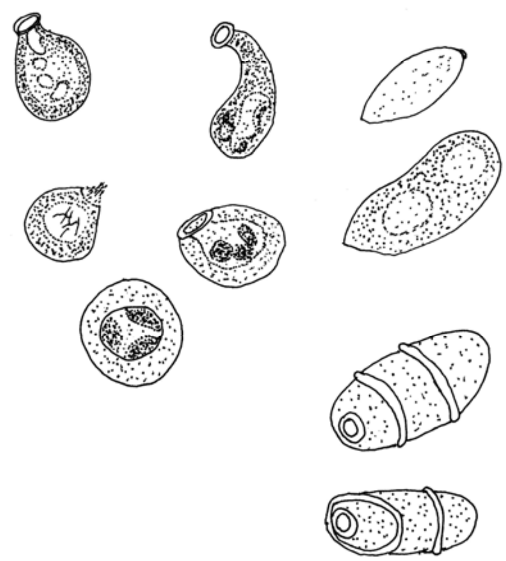

**Figure 9.** Mycroscopic characteristics of diferent cysts. a and b Apicomplexa like cysts; c, fuciform cyst; d, e, g, smal spherical cysts; f, large cyst with two large spores forming inside; h and y atipical shape cysts.

## DISCUSSION

Slides for microscopic observation were not intended for the study of the parasite, but for the study of gonad development. Therefore, the staining techniques did not allow for a clear interpretation of the different stages found. Even though, enough information is available to ascertain that a Coccidian *Apicomplexa* is infecting the digestive gland of *Strombus gigas* from San Andres and Alacranes reef. The various structures identified coincide with different stages of the life cycle of other Coccidians parasites from Vertebrates (Lom 1970, Levine 1985, Paperna and Lainson 2000) among others and from Mollusks (Perkins 1998, Gestal *et al*. 2002). As stated by some of these authors, *Apicomplexa* usually have a complex live cycle, including different hosts, but it is not unusual that the whole life cycle takes place within the same host. Apparently this is the case for the *Strombus gigas* parasite, but a specific study should be undertaken, given the apparent impact that it seems to have on the reproductive efficiency of the affected populations and its apparent wide distribution.

## ACKNOWLEDGMENTS

The ichthyology laboratory of CINVESTAV-IPN Mérida for its logistic support of this histology study and histological assistant Teresa Colás Marrufo.

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