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Morphology of the gastrointestinal tract of cascadura fish (*Hoplosternum littorale*) and its pathogenic fauna

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Abstract

Cascadura, (*Hoplosternum littorale*) is a delicacy in some countries; however, literature on this species is limited. This study aimed to identify gastrointestinal and hematological parasites and their potentially associated pathological effects in wild and farmed Cascadura in Trinidad. Samples of forty (40) wild and farmed Cascadura fishes were collected for this study. Morphometric measurements of whole fish and gastrointestinal tracts (GIT) were recorded. Blood smears were processed and stained with Wright's Giemsa and GIT sections stained with Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS). The gross anatomy and histological features of the GIT of the Cascadura were consistent with the fish belonging to the Callichthyidae family. Hematological parasites were found in 13 out of the 40 (32.5%) fishes (nine wild; four farmed): 6 of 13 belonged to *Alternaria* spp. (15%), 6 of 13 were protozoa (15%) and 1 of 13 (5%) was a blood fluke belonging to the family Aporocotylidae. Three gastrointestinal nematodes were found in three wild fish, one identified as an anisakid. Gastric and intestinal structures appeared normal; however, inflammatory cells were found in one wild fish with damaged villi and degenerated epithelium of the intestine.

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Introduction

The structure of the gut in the fish is a complex structure which is responsible for ingestion and digestion of the food as well as assimilation. The anatomy of the fish gut is important in guiding appropriate feeding strategies of the fish once cultured (1-3). The Cascadura, (*Hoplosternum littorale*) is a Neotropical, freshwater, armored catfish typically found in slow-moving rivers, streams, and swamps. They are facultative air breathers, inhabiting areas low in dissolved oxygen (4). Previous research has identified internal parasites in the Cascadura. In Trinidad, Cascadura also known as 'cascadoux' is consumed year-round as a protein source by locals and the tourists. They can be purchased fresh from roadside vendors or frozen at supermarkets or captured from water sources. Additionally, they can be used as aquarium fish, in research and in public education (5). Fish farming generates revenue and employment for those involved in the industry and provides rural communities with a source of income through subsistence farming. It also enriches the diverse culture of the region as part of its folklore (6). Fish pathogens cause clinical or subclinical disease that may result in economic losses due to mortalities, reduced production, and increased cost of treatment and low growth rate as well as it may has zoonotic threats, to human consumers (7). Wild fish can be reservoirs of parasites for aquaculture fish and vice versa (8).

There is limited research on this species; however, there is a growing interest in aquaculture of this species. As such, this study aimed to observe the histomorphology of the GIT to determine and document Cascadura parasites.

Materials and methods

Ethical approval

Ethical approval was granted by the University of the West Indies, Campus Ethics Committee, St. Augustine Campus (CREC-SA.0592/11/2020).

Fish Samples and experimental design

The sample size was calculated using the resource equation method (9). A sample of 20 farmed and 20 wild caught Cascadura fishes were collected from June - August 2021 from North-East and Central Trinidad (Figure1). The fishes were transported in containers filled with water from the fishes' environment, to the anatomy laboratory at the School of Veterinary Medicine, University of the West Indies. The fishes were weighed and their lengths were measured using a Schuler Scientific™ scale and a standard measuring tape respectively. Each fish was decapitated with scissors and blood was immediately collected by inserting a heparinized microcapillary collection tube into the pectoral articulation (10). Thin blood smears were made, air-dried, fixed, and stained with Wright's Giemsa. The slides were then examined under a light microscope using an oil immersion at 100x magnification. All fishes were sexed after dissection and recorded.

The length and weight of the intestine were recorded. Smears of the gastric and intestinal ingesta were made and observed for the presence of parasites using a light microscope. Grossly visible parasites were removed with forceps and visualized using a dissection microscope Also, viscera were collected in sterile sample cups, labelled, and fixed in 10% buffered formalin for 48 hours. Following fixation, they were processed and embedded in paraffin wax, then cut into sections at 3-4 micrometers using a microtome. Sections were stained with Hematoxylin and Eosin and Periodic Acid-Schiff (11-17) and then examined under a light microscope (Olympus BX 40™ with an Olympus DP 15™-megapixel digital camera, Japan).

Results

Gross anatomy and morphometry

The adult Cascadura displayed a cylindrical body with a flattened ventrum covered by two rows of dark brown to black overlapping bony plates. This fish has a single caudal, anal, dorsal, and adipose fin and paired pectoral and pelvic fins. The mouth was described as being inferior with no visible teeth and outlined by the presence of barbels (Figure 1). The esophagus was short and tubular, connecting to the cardiac stomach anteriorly, while the stomach was 'C' shaped and thick-walled. It comprised of the cardiac, fundic and pyloric regions. The intestine can be described as having an anterior, middle, and posterior section. The pyloric region communicates with the anterior intestine. Handling of the intestine revealed that the posterior portion of the intestinal wall was thinner than the anterior and middle segments (Figure 2). The statistical analyses that were done includes the means and standard deviations of the length of the whole fish, body weight, length of intestines and weight of intestines in wild and farmed cascadura which are shown in table 1. For farmed cascadura, the mean body length was 160.53 mm with a standard deviation of +/- 2.57 mm. The mean total body weight was 60.43 g with a standard deviation of +/- 22.16g. The average length of intestines was 145.33 mm with a standard deviation of +/- 44.02 mm. The average weight of intestines was 2.33 g with a standard deviation of +/- 1.00 g. For wild cascadura, the mean body length was 161.53 mm with a standard deviation of +/- 1.28 mm. The mean total body weight was 53.63g with a standard deviation of +/- 10.47 g. The average length of intestines was 140.93 mm with a standard deviation of +/- 22.27 mm. The average weight of intestines was 2.13 g with a standard deviation of +/- 0.63 g (Table 1).

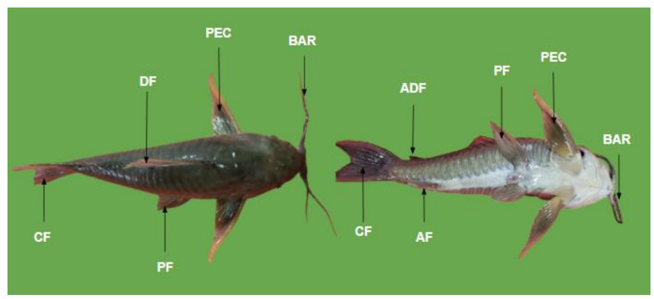

Figure 1: The external anatomy of the Cascadura (dorsal and ventral views). CF: Caudal fin; AF: Anal fin; DF: Dorsal fin; ADF: Adipose fin; PEC: Paired pectoral fin; BAR: Barbels; PF: Pelvic fin.

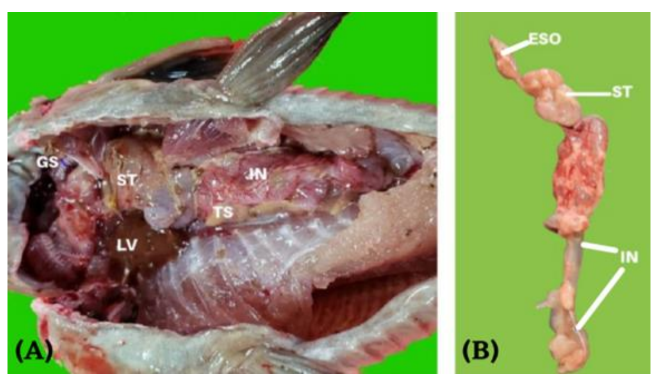

Figure 2: Topography of the GIT and associated organs of the Cascadura in situ (A and isolated organs of the GIT (B). GS: gills; ST: Stomach; LV: Liver; TS: Testes; IN: Intestine; ESO: Esophagus.

Table 1: Mean and standard deviation of gross measurements of farmed and wild Cascadura

Measurement	Farmed Cascadura		Wild Cascadura	
	Mean	Standard deviation	Mean	Standard deviation
Body Length	160.53mm	2.57	161.53mm	1.28
Total body weight	60.43g	22.16	53.63g	10.47
Length of intestines	145.33mm	44.02	140.93 mm	22.27
Weight of intestines	2.33g	1.00	2.13g	0.63

Histological features

The cardiac, fundic and pyloric regions of the stomach varied from each other by the arrangement of the mucosal layer and the absence of gastric glands in the pyloric region. The stomach was organized into mucosal, glandular, and submucosal layers respectively. The mucosal layer presented with simple columnar epithelium. The gastric mucosal layer comprised of epithelial cells containing neutral glycoproteins which stained purple with PAS. This allowed for differentiation of each segment of the stomach according to the number of gastric glands making up each portion of the stomach. The submucosal layer comprised of loose connective tissue with collagen fibers interspersed with blood vessels. The mucosa increased in height and number of disordered folds from cardiac to pylorus while the submucosa increased in thickness (Figure 3).

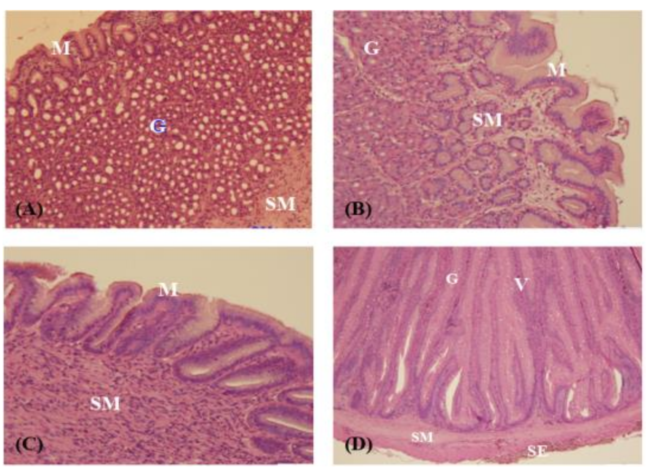

Figure 3: The stomach and intestine of Cascadura fish. A: Cardiac region (H&E x20); B: Fundic region (H&E x40); C: Pyloric region (H&E x40); D: Anterior intestine (H&E x10).

The intestine was divided into the proximal, middle, and distal intestine, with layers presented as mucosa, submucosa, muscular, and serosa respectively. The mucosa was longitudinally oriented with villi decreasing in height from proximal to distal intestine. Alternatively, the goblet cells increased in size nearing the distal intestine. The mucosal layer was presented with simple columnar epithelium with a brush border and goblet cells while the cytoplasm of the goblet cells contained mucos substances. The submucosa consisted of dense connective tissue and blood vessels. The serosa comprised of dense connective tissue surrounded by a layer of mesothelial squamous cells and blood vessels. The muscular layer presented with two smooth muscle layers arranged in longitudinal and circular orientation from external to internal respectively (Figure 3).

Pathogenic fauna

Four wild Cascaduras were found with trematodes in their stomachs (Table 2 and Figure 4). One wild fish was found with a trematode encysted in the submucosa of the fundic region of the stomach (Figure 5). There was an influx of inflammatory cells surrounding the cyst. Two were found with a single trematode in the pyloric regions, while the fourth fish contained three trematodes in that region (Figure 5). All farmed fish found possessed intact villi and epithelium with no parasites observed in the stomach. A trematode was observed in the lumen of the anterior intestine of one wild Cascadura, but the submucosa, muscular and serosal layers were intact (Figure 5). There was also an influx of inflammatory cells surrounding the trematode. One farmed fish possessed damaged villi and degenerated intestinal epithelia. Closer examination revealed the fibrous capsule of an excysted trematode.

Table 2: Parasitic prevalence in the gastrointestinal tract, blood and tissues of wild and farmed cascadura

Technique	Parasite	Parasites Observed	Wild Fish [n(%)]	Farmed Fish [n(%)]
Fecal Examination	Nematodes	Family Anisakidae	1 (5)	0 (0)
		Unidentified spp. A	2 (10)	0 (0)
Histology	Trematodes	Unidentified spp. B	4 (20)	1 (5)
Blood Smears	Trematodes	Aporocotylidae gen.sp	0 (0)	1 (5)
	Protozoa	Intraerythrocytic Inclusion bodies	4 (20)	2 (10)
	Fungi	*Alternaria* spp.	5 (25)	1 (5)

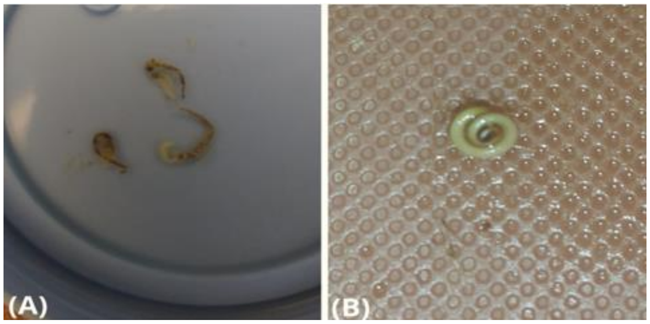

Figure 4: Unidentified nematode found in the intestine (A) and an Anisakid Nematode found in the stomach (B) of a Wild Cascadura.

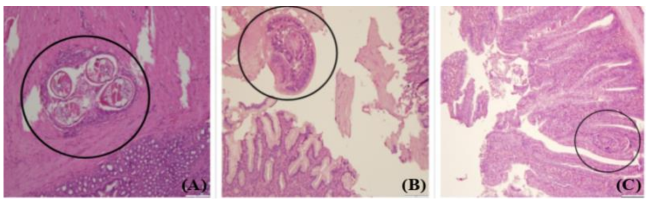

Figure 5: The stomach and intestine of Cascadura fish containing a trematode. A: Fundic region (H&E x20); B: Pyloric region. (H&E x20); C: Anterior intestine (H&E x10).

Blood smears

As shown in table 2, a blood fluke belonging to the family Aporocotylidae was found in 1 (5%) wild fish. Intraerythrocytic inclusion bodies were found in 4 (20%) wild fish and 2 (10%) farmed fish. A fungus, *Alternaria* spp. and its fungal spores were also seen in 5 (25%) wild fish and 1 (5%) farmed fish (Figure 6).

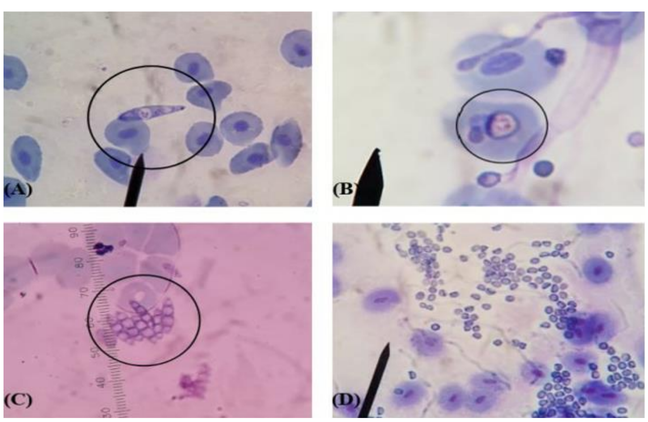

Figure 6: Photomicrographs of the organisms found in the blood smears. A: Aporocotylid (blood fluke). *Scale bar* = 100µm); B: Intraerythrocytic inclusion bodies (Protozoa. *Scale bar* = 100µm). C: *Alternaria* (fungus -*Scale bar* = 100µm); D: Fungal spores released from *Alternaria* (*Scale bar* = 100µm).

Discussion

The Cascadura has many notable structural features such as the barbels, which function as sensory organs that aid the fish in finding food (18). The Cascadura is sexually dimorphic. Males are larger in size and have prominent hook-shaped spines attached to the dorsal aspect of their pectoral fins, while females are smaller with less prominent, straight spines (19). These findings were also observed in this study.

Exploration of the GIT of Cascadura showed that the gross anatomy was consistent with fish belonging to the Callichthyidae family. It comprised the oral cavity, esophagus, stomach and intestine. Handling of the intestine revealed that the posterior portion of the intestinal wall was thinner than the anterior and middle segments. This can be attributed to the Cascadura being a facultative air-breather. It takes in air from the water's surface in hypoxic conditions, this air then goes through the GIT and to the intestine where gaseous exchange occurs across the thin wall of the posterior intestine. This area of the intestine provides a short air-blood diffusion distance. The intestine is also highly vascularized, allowing quick and effective gaseous exchange (20).

The stomach of the Cascadura can be divided into cardiac, fundic and pyloric regions. Histologically, the stomach was found to have a high number of gastric glands which is a typical feature of omnivorous fish species within this family. This can be attributed to them having an increased need to digest protein-rich material such as algae, detritus and crustaceans. It also acts as a protective layer against microorganisms by being highly acidic. Compared to the cardiac and fundic regions of the stomach, the gastric glands were sparse in the pyloric region. The reason for this modification was that this region stored food before passing it to the intestine, while the other two regions were focused on digestion. The gastric glands stained positive for PAS which indicated the presence of neutral glycoproteins (21-23).

The Cascadura's intestine is divided into three regions- the anterior, middle, and posterior parts. At the histological level, the lumen of the intestine was lined by extensive villi and goblet cells which functioned to aid in further digestion and absorption of ingesta. Goblet cells, stained positively with PAS, indicated the presence of mucopolysaccharides. Furthermore, the goblet cells increased in number and size from anterior to posterior intestine serving to protect the intestinal lining and aiding in the expulsion of fecal matter (3,24).

Parasitic organisms are common in fish from wild populations where there is a diverse aquatic environment. Wild fish can therefore serve as intermediate hosts or transmit parasites to farmed/cultured fish when introduced to fish farms. The sampling areas where the fishes were collected had similar conditions which are favorable for the spread of parasites. After the consumption of parasites by the fish, they interact with the hosts' cells by ingestion or attachment, growth, multiplication, senescence and release of infectious stages. The host's immune system responds to helminths by creating a granulomatous inflammatory response in which the host's immune system encapsulates the parasite in an attempt to isolate and destroy it (25). This phenomenon explained the histological and morphological changes within the GIT of the Cascadura.

Anisakid nematodes (Family: Anisakidae appeared in the current study in one adult wild cascudra fish; similar to that reported in two species of marine fish northwest Arabian gulf (26). They are parasitic worms with notable genera including Anisakis, Pseudoterranova, Hysterothylacium and Contracaecum spp. In freshwater environments, humans are likely to be the accidental hosts of these parasites. Copepods are included in the diet of adult and juvenile Cascadura; this suggests the most likely mode of infection. Cascadura, like other fish, act as intermediate hosts to third stage (L3) larvae. These nematodes cause anisakiasis in humans (24,27). In humans, ingestion of raw or undercooked fish products, e.g. anchovies, infected with Anisakis spp. can result in nausea, vomiting, abdominal distension, mild to severe anaphylactic reactions and hematochezia. Despite this, nisakiasis is still underdiagnosed (28).

Histologically, all the unidentified trematodes were found in either the fundic or pyloric stomach or intestine. It may be suggested that the area was ideal for habitation due to a lack of proper defenses; the pylorus was non-glandular, and the intestines had fewer gastric cells. According to Lacerda *et al.* (29), known trematodes of the Cascadura are *Crassicutis intermedius*, *Herpetodiplostomum caimanicola*, *Kalipharynx* sp. (Fellodistomidae: adult), and *Magnivitellinum corvitellinum*. *Magnivitellinum corvitellinum* is known to reside in the intestine. More research is needed to identify these trematodes and determine their effects on Cascadura and humans since no literature has been recorded to date.

The aporocotylids (Family: Aporocotylidae) are freshwater and marine blood flukes that infect wild and farmed fish, however, farmed fish appear to be more susceptible (30). They reside in the host's circulatory system such as in branchial vessels and the heart. Their eggs may be found in the branchial filaments. The fluke can have either a direct or indirect life cycle. The indirect life cycle of some marine aporocotylids includes the use of terebellid polychaetes (*Nicolea gracilibranchis*, *Longicarpus modestus*, *Reterebella aloba* and *Terebella* sp: *Neoamphitrite vigintipes*) as intermediate hosts.

The direct life cycle involves direct penetration by cercariae into unsuspecting hosts. This is considered the dominant route of infection in fish. The fluke can be free-living in the environment; however, it is difficult to detect. This is the most probable cause of infection in these farmed fish as the organism was present in the environment. There is no defined name for the illness; however, it can be caused by the accumulation of eggs within the gill filaments. Infected fish may experience gill hyperplasia, and egg encapsulation in the gills and ventricles. Papillae form due to endothelial proliferation in the afferent branchial arteries. Hatching miracidia may cause multiple lesions such as microhemorrhages which potentially trigger an inflammatory response and result in anemia (29). Further research, relating to the zoonotic potential of aporocotylid flukes is warranted.

The unidentified intraerythrocytic inclusion bodies were found to be protozoa which can potentially cause clinical signs ranging from a mild anemia to severe pathological changes depending on the parasite burden (31).

Alternaria spp. is a ubiquitous fungal plant pathogen (32). There is no published literature stating its effects on Cascadura, but it is known to affect other fish species. *Alternaria* spp. cause phaeohyphomycosis, along with *Cladosporium herbarum*, *Chaetomium globosum*, *Cadophora luteo-olivace*a, *Penicillium* sp, *Phoma herbarum*, *Pseudophacidium ledi,* and *Valsa sordida,* in saffron cod and rainbow smelt according to Meyers *et al*. (33). Following exposure to adverse environmental conditions, a cerebral infection in carps and a fatal behavioral disorder may result. Infection occurs due to microbial contamination, but it does not usually result in disease. Environmental stress may increase the chances of infection and fish may display signs of illness. A lack of good aquarium keeping in fish farms increases the chances of fungal infection in fish (34).

The current study did not indicate skin lesions; however, fish infected with *Alternaria* spp. may display large black, oval, external lesions of the skin and smaller foci on the gills. These lesions occur commonly during the late fall and early winter months of October through December. Invasion of internal tissues is rare, yet it seems to occur in subsistence fish farms in Saffron cod (33). Transmission is unknown but it is suggested to occur by ascospores contained in ambient seawater or sediments, increased by rain, flooding and stress that require previous mechanical tissue injury as a portal of entry into the host. Hattab *et al*. (35) stated that although it rarely causes human infection, it can cause cutaneous lesions and rhinosinusitis in immunocompromised and healthy individuals. A study, conducted in 2015, described in detail, patients who developed *Alternaria*-related cutaneous lesions after administration of immunosuppressant medications following a kidney transplant (36).

Based on the findings in this study, there is a great need to identify the parasites present in wild and farmed Cascadura in Trinidad. It is imperative that the potential pathological effects that these parasites have on such fish and human consumers be investigated to provide healthy fish and improve productivity and profitability in aquaculture systems.

Conclusion

The gross anatomy and histological features of the GIT of the Cascadura are consistent with fish belonging to the Callichthyidae family. Parasites found in this study included trematodes, nematodes, protozoa and fungi, several of which are of veterinary and public health importance (e.g., *Alternaria* spp. and *Anasakis* spp). Histological changes were observed due to the presence of flukes in the GIT causing epithelial degeneration and villi detachment. Future studies can include conducting similar research using a larger sample size and researching and identifying the unknown species of parasites found in the Cascadura, its zoonotic potential and mode of transmission. Parasitism in the musculoskeletal system of the Cascadura and in other local fish species such as the Guabine (*Hoplias malabaricus*) could also be examined.

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Conflict of interest

There are no conflicts of interest.

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دراسة شكلية للجهاز الهضمي لأسماك كاسكادورا (*Hoplosternum littorale*) وكائناتها المسببة للأمراض

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الخلاصة

الكاسكاديورا، (*Hoplosternum littorale*) من اكثر الأسماك شيوعا في بعض البلدان، ومع هذا فان المصادر العلمية عن هذا النوع محددة. هدفت هذه الدراسة الى التحري عن الطفيليات المعدية المعوية والدموية والتاثيرات المرضية المرتبطة بها في اسماك الكاسكاديورا في المسطحات المائية والمستزرعة في ترينيداد. لغرض اجراء هذه الدراسة جمعت أربعون (٤٠) عينة من اسماك الكاسكاديورا من المسطحات المائية والمستزرعة. سجلت القياسات الشكلية للطول الكلي للاسماك وللقناة المعدية المعوية. حضرت المسحات الدموية وصبغت بالرايت والكيمزا وتم صبغ المقاطع المعدية المعوية بالهيماتوكسلين والايوسين. والملون الحامضي بيريوديك شيف. كان التشريح العياني والصفات النسجية للقناة المعدية المعوية متسقة مع الاسماك التي تنتمي الى عائلة Callichthyidae. شخصت الطفيليات الدموية في ١٣ سمكة من اصل ٤٠ (٣٢,٥%) (تسعة من المسطحات المائية وأربعة من المستزرعة): ٦ من ١٣ (١٥%) تعود للانواع Alternaria، ٦ من ١٣ (١٥%) اوالي و١ من ١٣(٥%) كانت لديدان الدم التي تعود لعائلةAporocotylidae . تم تسجيل ثلاثة أنواع من الديدان الخيطية المعدية المعوية في ثلاث اسماك من المسطحات المائية، كانت واحدة منها هي للنوع anisakid. عيانيا لم يكن هنالك تغييرات مرضية عيانية في كل من المعدة والامعاء مع وجود تغييرات مرضية نسجية في امعاء سمكة واحدة شملت ارتشاح الخلايا الالتهابية وتحطم الزغابات والتنكس الفجوي في ظهارة الأمعاء.