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Acanthocephalan and Trematode Endoparasites in Rabbitfish *Siganus fuscescens* from the Selected Coastal Areas of Surigao City, Surigao del Norte, Philippines

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ABSTRACT

The rabbitfish, *Siganus fuscescens*, is an economically important fishery in Surigao City, Philippines. This study was conducted to assess the zoonotic potential of the endoparasites infecting *S. fuscescens* collected from September to November 2021 in the three selected coastal areas of Surigao City, Surigao del Norte, Philippines. Isolated worms were identified using light microscopy and appropriate staining techniques based on their morphological characteristics. Two endoparasite species were recovered from the intestine of the siganids, the acanthocephalan *Sclerocollum rubrimaris*, and the trematode *Hexangium sigani.* Single infections of *S. rubrimaris* were common among fish samples, and an overall prevalence of 45% and a mean intensity of 30±23.34 (N=150) were recorded. The fish length (rs= -0.243) and weight (rs= -0.139) had a significant but weak negative correlation with the parasite load. With prevalence of 100%, results revealed that the Canlanipa station has a significantly higher prevalence of infection (100%) compared to Perlas (14%) and Bilang-bilang (22%). The endoparasites are not zoonotic, yet, intestinal hemorrhage was observed in fish with heavy infections with *S. rubrimaris*. This study is the first report of endoparasite infection in siganids of Surigao. A survey of endoparasites in other fish species in the coastal waters concerning water quality and heavy metal pollution is recommended.

Keywords: *Siganus fuscescens, Sclerocollum rubrimaris, Hexangium sigani, endoparasites*

1 Introduction

The Philippines is among the top fish-producing countries worldwide (Lamarca 2017) and is home to about 60 percent of the world's known fishes. Among these are the herbivorous siganids or rabbitfishes widely distributed in Indo-Pacific regions (El-Dakar et al. 2011). In many Southeast Asian nations, siganids are aquacultured due to their nutritional value (Wahyuningtyas et al. 2017) and good market demand (El-Dakar et al. 2011). Fisheries in the country significantly contribute to the national economy and food security (Santos et al. 2011); hence, parasite infection is an impending threat that may directly or indirectly affect fish reproduction, growth, and survival (Quiazon 2015). Several species of rabbitfish, such as *Siganus fuscescens*, harbor a diverse parasite community (Quilichini et al. 2011; Shih and Jeng 2002).

Upon ingestion of improperly cooked infected fish, humans may become accidental hosts of endoparasites (Praveen et al. 2015). Fish-borne parasitic zoonoses are more prevalent in developing countries and may be attributed to their traditional preparation of fish dishes (Jithendran 2018). Anisakid nematodes, for instance, are widely known to infect commercially important fishes and humans, causing clinical manifestations such as gastrointestinal disorders and allergic reactions (Ramos et al. 2020). Moreover, among the major groups of parasites, trematodes and acanthocephalans are known to infect fish. In siganid species, the intestinal trematode *Hexangium sigani* (Khalifa et al. 2018) and the thorny-headed worm *Sclerocollum rubrimaris* (Hassanine and Al-Hasawi 2021) are two of the many endoparasites that were isolated. Parasites have detrimental effects on their fish hosts. Heavy infections can cause mechanical, physiological, and reproductive damage that might, later on, lead to death (Iwanowicz 2011). Nonetheless, recent studies suggest that endoparasites can benefit their marine hosts. The acanthocephalan *S. rubrimaris* could lessen the harmful effects of trace metal pollution on their siganid host, *Siganus rivulatus,* inhabiting the polluted waters of the Red Sea, Egypt (Hassanine and Al-Hasawi 2021).

Zoonotic potential of endoparasites in rabbitfish of Surigao City is not yet assessed. Hence, the findings of this study can contribute to the body of knowledge, providing empirical data to better understand siganids and their parasites in Philippine coastal waters.

2 Materials and Methods

Study Area

The study was conducted in three selected locations along the coastal areas of Surigao City. The municipality faces the Pacific Ocean, and among the mainland coastal barangays, only two were selected for this undertaking (Figure 1). The selection was merely based on the occurrence of siganids populating the sites. In each location, 50 fish samples were collected in a span of two months following the residential coastal area of Purok-3 Barangay Canlanipa (9°46’37”N, 125°29’47”E) and the seaports of Punta Bilang-bilang (9°46’52”N, 125°29’48”E) and Purok Perlas (9°46’51”N, 125°29’47”E) of Barangay Taft.

Fish collection and parasite identification

Fish samples were collected from September to November 2021. Rabbitfishes were caught using a fishing net in Barangay Canlanipa. At the same time, representatives from Punta Bilang-bilang and Purok Perlas of Barangay Taft were collected using fish hooks with the assistance of a local fisherman. Captured fish samples were then immediately examined to isolate and document live endoparasites or frozen for future dissection. Length (cm) and weight (g) were measured before dissection using a digital Vernier caliper and an electronic balance.

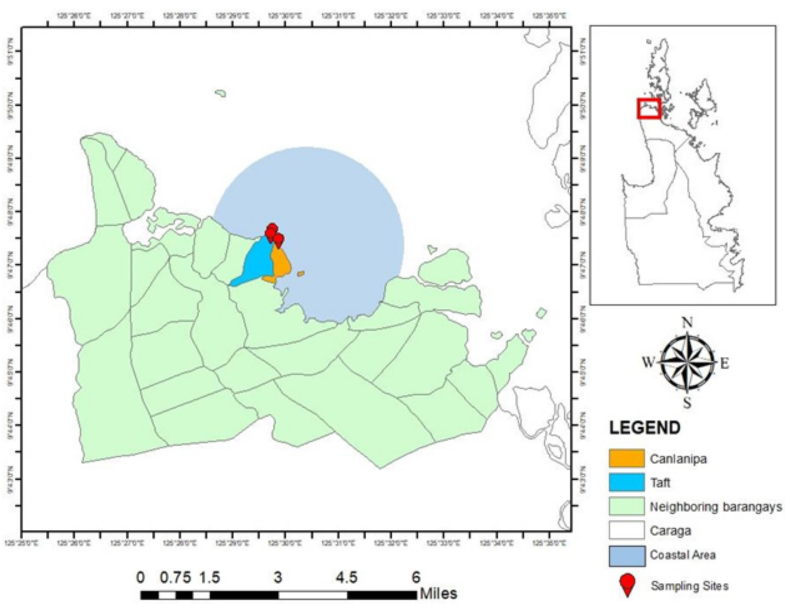

Figure 1. Map showing the selected sampling sites of Surigao City, Surigao del Norte, Philippines.

Individuals were inspected for morphological damage due to endoparasite infection.

A total of 150 fish samples were dissected. The gastrointestinal tract was removed from the body cavity and placed on a Petri dish with distilled water. Specimens were examined using a corded motic microscope (ecoline C-10 LED) aided by a motic camera. Visually spotted parasites were isolated, transferred to a separate dish, and counted. Afterward, endoparasites were washed with distilled water and preserved in vials with 70% ethanol (Upton 2005).

Acanthocephalans were kept unstained and were directly viewed under the microscope, documenting their anterior and posterior part, particularly the proboscis and other conspicuous organs relevant for proper identification. Trematodes were stained using an acetocarmine stain for 2 h. The endoparasites were then exposed to 70% ethanol to halt staining and later destained briefly in 70% acid ethanol. These were eventually blued up in 70% basic ethanol and dehydrated in a series of alcohol concentrations.

Data Analyses

The prevalence rate of acanthocephalan and trematode endoparasites (No. of infected fish / total number of examined fish X 100) and Mean intensity of endoparasite infection (Total n of isolated parasites/ total number of infected fish samples) were computed (Dela Cruz et al. 2022). Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) (Hermida et al. 2012). Mann-Whitney U and Kruskal Wallis tests were accomplished to compare the infection rate between sampling areas. While Spearman correlation analysis was utilized to determine the relationship between fish weight and length and the parasite mean intensity.

3 Results and Discussion

Of the 150 dissected fish samples, 68 (45%) were infected with endoparasites. The Canlanipa area had the highest overall prevalence (100%), followed by Punta Bilang-bilang (22%) and Purok Perlas (14%). 2,016 endoparasites (mean intensity of 30±23.34) were isolated from fish samples across sampling sites. Canlanipa had the highest mean intensity of 37±27.81, followed by Bilang-bilang (14±7.38), and Perlas (5±2.27).

Acanthocephalan and trematode endoparasites isolated

Two endoparasite species were recovered from the examined rabbitfish samples: the acanthocephalan *Sclerocollum rubrimaris* (Figure 2) and the trematode *Hexangium sigani* (Figure 3). Identification of the two parasite species was based on their conspicuous morphological features, as described in various reputable journal articles (Dzikowski et al. 2003; Hassanine 2006; Eldeen et al. 2014; Hassanine et al., 2016; Khalifa et al. 2018; Hassanine and Al-Hasawi 2021). These parasites were found and isolated from the siganid hosts' intestines. Predominantly, fish samples exhibited single infections with *S. rubrimaris* (45%, n=68); and occasionally, concurrent infections with both *S. rubrimaris* and *H. sigani* (3%, n=5).

Both male and female unstained acanthocephalan were examined. Several defining features were observed that are typically possessed by *S. rubrimaris*, known to parasitize several siganid species (Hassanine 2006; Eldeen et al. 2014). The isolated acanthocephalan have unspined tegument; relatively short, cylindrical proboscis armed with alternating rows of sharp hooks; lemnisci slightly longer than the receptacle; with oval-shaped male testes—anterior testis almost equatorial—and well-developed copulatory bursa (Hassanine 2006). This is the first record of *S. rubrimaris* among siganids in Philippine waters.

In fish samples with intense infection of *S. rubrimaris,* parasite obstruction resulted in hemorrhage in the infected area, apparently attributed to the attachment of the acanthocephalans' proboscis to the intestinal wall. This observation was reported by Eldeen et al. (2014) in *Siganus rivulatus* infected with *S. rubrimaris*, further indicating that apart from intestinal damage, parasite obstruction also impedes the movement of digested materials as well as the absorption of nutrients. Moreover, the trematode was rarely isolated among the dissected fish samples; hence, no attributable morphological damage was detected. Morphological examination identified the endoparasite as *Hexangium sigani,* as described by Khalifa et al. (2018). Newly isolated trematodes were fleshy with white and smooth tegument; body dorsoventrally flattened and elongated; well-developed pharynx; uneven left and right ceca; oval-shaped anterior and posterior testes; very prominent uterus situated medially; and V-shaped

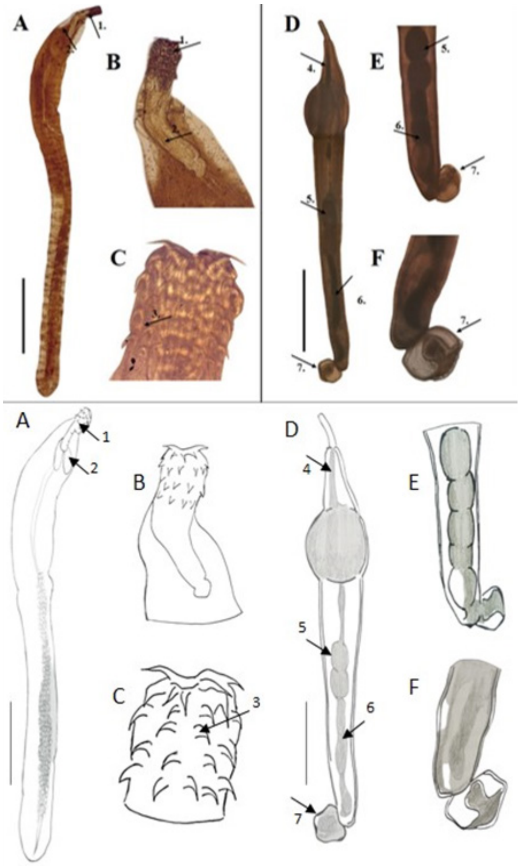

Figure 2. Photomicrograph and line drawing of *Sclerocollum rubrimaris* isolated from *Siganus fuscescens* inhabiting the three selected coastal areas of Surigao City, Surigao del Norte, Philippines. A (under scanning objective 40x): Unstained female Acanthocephala; B (under LPO 100x): Anterior end of the female endoparasite; C (under HPO 400X): Endoparasites' proboscis; D (under scanning objective 40x): Unstained male acanthocephalan; E (under scanning objective 40x): Enlarged image of the male endoparasites' posterior end; F (under LPO 100x): Posterior end of the male endoparasite. Arrows pointing 1. Proboscis, 2. Receptacle, 3. Hook, 4. Lemniscus, 5. Testis, 6. Cement gland, and 7. Bursa. Scale bars = 1 mm (A), 2 mm (D).

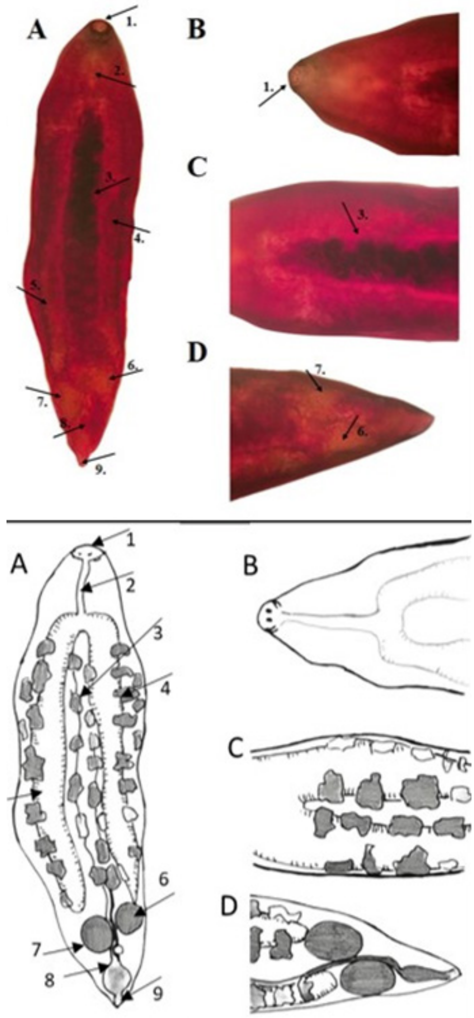

Figure 3. Photomicrograph and line drawing of *Hexangium sigani* isolated from *Siganus fuscescens* inhabiting the three selected coastal areas of Surigao City, Surigao del Norte, Philippines. A (under scanning objective 40x): Whole trematode endoparasite stained with acetocarmine stain; B (under LPO 100x): Endoparasites' anterior end; C (under LPO 100x): Endoparasites' mid-body part; D (under LPO 100x): Endoparasites' posterior end. Arrows pointing 1. Pharynx, 2. Esophagus, 3. Uterus, 4. Vitelline follicle, 5. Cecum, 6. Anterior testis, 7. Posterior testis, 8. Ovary, 9. Excretory vesicle. Scale bar = 1 mm.

excretory vesicle. To date, there were no known records of *S. rubrimaris* and *H. sigani* accidentally infecting humans.

Fish length and weight in relation to helminth intensity

The relationship between fish length and endoparasite intensity was determined (Table 1). Fish length ranges between 7.83 cm-14.34 cm (with a mean value of 10.27±1.05). Results revealed a significantly weak, negative association between fish length and *Sclerocollum rubrimaris* intensity (rs= -0.242; p=0.003). On the other hand, *Hexangium sigani* intensity has a weak positive association and is not significantly correlated (rs= 0.001; p=0.996). The relationship between fish weight and endoparasite intensity was obtained (Table 2). The intensity of *S. rubrimaris* has a significant yet very weak, negative correlation with the fish weight (rs= -0.139; p= 0.090). *H. sigani* intensity has a weak, positive association (rs= 0.054; p= 0.508).

The negative correlation of the fish length and weight with *S. rubrimaris* intensity suggests that the parasite infection may inversely influence the fish's physical parameters. However, the associations were too weak, indicating that despite the substantial number of endohelminth isolated, it scarcelyaffected the length and weight of the fish samples. Given that their physical parameters were not adversely affected, it can be deduced that the examined siganids appear to tolerate helminth infection (Eldeen et al. 2014).

Acanthocephalan infection is not entirely detrimental to their host since, remarkably, *S. rubrimaris* can absorb and bioaccumulate toxic heavy metals, such as cadmium and lead (Hassanine and Al-Hasawi 2021). Frequent consumption of heavy metal-contaminated fish may lead to impaired mental and central nervous system function and harm the lungs, kidneys, and other vital organs (Isangedighi and David 2019). Therefore, completely eradicating *S. rubrimaris* from polluted waters is not advisable since they can help accumulate toxic heavy metals better than their hosts (Al-Hasawi 2019). Consequently, it can then be assumed that the helminths will not threaten the local fisheries as long as the infection rate is not high enough to elicit harmful effects on the siganid hosts.

A comparison of endoparasite infection across sampling areas indicates a significant difference in the intensity of *Sclerocollum rubrimaris* (p=0.001). Canlanipa has a significantly higher infection than the other locations in terms of acanthocephalan and trematode intensity. Purok Perlas and Punta Bilang-bilang are located in the same barangay, which could explain why there was no significant difference between the two sampling sites. It is assumed that the rabbitfishes populating these areas may have overlapping habitats and share almost similar diets. Also, the seaports in Perlas and Bilang-bilang were recently reconstructed, which might have disturbed the natural habitat of the

Table 1. Correlation between the fish length and the intensity of the endoparasites isolated from *Siganus fuscescens* in the three selected coastal areas of Surigao City, Surigao del Norte, Philippines.

Intensity	*Fish Length* Correlation coefficient (r_s)	p-value	Remarks
S. rubrimaris	-0.242*	0.003	Significant
H. sigani	0.001	0.996	No Significant
Total helminth load	-0.243*	0.003	Significant

*Significant at 10% alpha; Tested in Spearman's Rho

Table 2. Correlation between the fish weight and the intensity of the endoparasites isolated from *Siganus fuscescens* in the three selected coastal areas of Surigao City, Surigao del Norte, Philippines.

Intensity	*Fish Weight* Correlation coefficient (r_s)	p-value	Remarks
S. rubrimaris	-0.139*	0.090	Significant
H. sigani	0.054	0.508	No Significant
Total helminth load	-0.139*	0.090	Significant

*Significant at 10% alpha; Tested in Spearman's Rho siganids found in the coastal areas, affecting their source of nourishment and the potential for helminth infection.

The presence of amphipod hosts (Al-Jahdali et al. 2015) and aquatic vegetation (Hassanine et al. 2016) in the marine environment have roles in the life cycle of both the acanthocephalan and trematode worms. Rabbitfish larvae consume zooplankton (Quinitio and Sa-an 2008), whereas the mature ones typically feed on algae (Nanami 2018). Although Canlanipa is a residential area and is just adjacent to Perlas, the site is relatively undisturbed, which could allow the isolated endoparasites, *S. rubrimaris* in particular, to flourish and continuously infect its siganid host. The helminth's uninterrupted life cycle elucidates the significantly high infection rate in the said location.

4 Conclusion and Recommendations

This study provides relevant information on *Sclerocollum rubrimaris* and *Hexangium sigani* infection in *S. fuscescens* collected in the selected coastal areas of Surigao City. The endoparasites recovered are not recognized as zoonotic. However, heavy infection of the acanthocephalan endoparasite caused intestinal damage in the fish examined. Molecular analysis is necessary to identify the isolated endoparasites accurately. With the emerging studies related to *S. rubrimaris* and its ability to bioaccumulate toxic heavy metals from the intestine of its siganid host, it is recommended for future undertakings to obtain water, parasite, and fish tissue samples for heavy metal analyses. Findings will be vital to assess the potential of this host-parasite relationship to biomonitor heavy metal pollution in the coastal areas of Surigao City.

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Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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