

Histopathological Analysis of Trypanorhyncha (Cestode) Larvae (Plerocercoid) Infestation in the Intestine of *Scomberomorus commerson* (Lacepede, 1800)

*Mumthaz TMV¹, Preethakumari VM², Sapna Jacob³, Mini PV⁴, Athulya K²,

¹Department of Zoology, Sir Syed College, Taliparamba, Kannur University, Kerala, India

²Department of Zoology, Sree Narayana College, Kannur University, Kerala, India

³Department of Zoology, Payyannur College, Payyannur, Kannur University, Kerala, India

⁴Department of Zoology, Govt. College, Kasargod, Kannur University, Kerala, India

Corresponding author: drmmumthaztmv@gmail.com

Abstract

Trypanorhyncha are common cestode parasites infecting marine fishes, but studies on these cestodes in the fishes of the Malabar Coast are limited. This study presents the first report of Trypanorhyncha in the southwest coast of India, focusing on their infestation in the commercially important fish, *Scomberomorus commerson*. The prevalence of infection was found to be 85.2%, with plerocercoid larvae encysted in the intestine. Histological examination revealed significant pathological alterations, including hyperplasia and atrophy of the intestinal epithelium mainly due to the immune responses of the host rather than feeding activities of the parasites. Microscopic analysis showed numerous cestode cysts with associated inflammatory responses, which likely impair nutrient absorption. The high prevalence of Trypanorhyncha infection in *S. commerson* provides insights into the host response and the behavior of the parasite within host tissues, highlighting the importance of monitoring and managing parasitic infections in marine fisheries.

Keywords: Trypanorhyncha, Cestoda, Plerocercoid, Histology, *Scomberomorus commerson*.

1. INTRODUCTION

Among the cestode parasites infested on marine fishes, members of Trypanorhyncha genera are very common, having a complex life cycle involving three hosts (de Sales-Ribeiro et al. 2021). Their cycle begins with elasmobranch fish: the definitive hosts, releasing free-swimming larvae: coracidia, which are ingested by small crustaceans: the first intermediate hosts, where they develop into proceroids. Following the consumption by teleost fish or invertebrates: the second intermediate hosts, the proceroid larvae migrate through the intestinal wall and become encapsulated within the host tissues, particularly in the viscera or muscles, where they develop as plerocercoid larvae before transmission to the definitive host (Overstreet, 1978; Alvarez et al. 2006; Haseli et al. 2010; Beveridge et al. 2014). These parasites demonstrate lower host specificity during their larval stages, allowing them to infect a wide range of hosts and facilitating widespread distribution from brackish to deep-sea waters, with notable species richness observed in tropical marine coastal habitats across the Indo-Australian area (Palm and Cairns 2008; Palm et al. 2009). The occurrence of plerocercoids in the intestine of commercially important fishes such as *Scomberomorus commerson*, underscores their ecological and economic impact within Indo-Pacific fisheries (Randall 1995).

Building on their intricate life cycle, the custodian order Trypanorhyncha Diesing (1863) members are significant yet often overlooked parasites in teleost fish. Despite the challenges in their taxonomy, these parasites are identifiable through distinct morphological features, particularly in their larval stages, which share identifiable scolex characteristics with adults. "The scolex, bearing 2 or 4 bothria (muscular suction grooves) with a tentacular apparatus. This apparatus has four retractile tentacles that can be everted and retracted using a rhyneal apparatus (consisting of tentacle sheaths and retractor muscles). Each tentacle is bearing hooks with species specific arrangement patterns, and are supported by four muscular bulbs that enable coordinated the mechanism for tentacle movement. This unique apparatus is used to anchor the parasite to the host's intestinal mucosa". The specific structure of the tentacles and bulbs is taxonomically important and serves as the key diagnostic features for identification of its adult tapeworms (Dollfus, 1942; Jones et al. 2004). Over the years, extensive taxonomic work has identified 254 species within this order (Palm 2004), with subsequent studies adding 23 more, bringing

the total to 277 valid species (Beveridge and Campbell 2005; 2006; 2007; Friggens and Duszynski 2005; Beveridge and Justine 2006; 2007; Campbell and Beveridge 2006; 2007; and Palm et al. 2009). Extensive taxonomic studies on larval trypanorhyncha have been carried out in various regions globally: the Gulf of California, Guinea and Arabia, Southeast Asia, Australia, and parts of the Red Sea, indicating their wide distribution (Overstreet 1977; Palm 2000; Ibrahim 2000; Friggens and Duszynski 2005; Jacob and Palm, 2006; Beveridge & Justine, 2007; Malek et al. 2007; Abdou and Palm 2008; Jensen 2009; Haseli et al. 2010; Ogawa et al. 2012; Schaeffner and Beveridge 2014; Beveridge et al. 2014; Palm and Bray 2014; Al-Zubaidy and Mhaisen 2021). However, the present work represents an initial record on the Trypanorhyncha infestation on commercially high valued host fish, *Scomberomorus* sp. of India's southwestern coast and highlighting the importance of further research into their distribution and host-parasite interactions along this region.

Scomberomorus commerson (Lacepede 1800) (Narrow-barred Spanish mackerel), belongs to the family Scombridae, is a highly valued edible fish with significant market importance along the Malabar Coast. This species is marketed fresh, dried, or salted, any threat to its population could significantly affect local fishing industry. *S. commerson* is an epipelagic and oceanodromous fish (Riede 2004), occurring across a range of marine habitats from offshore shelf regions to nearshore coastal waters at depths up to 200 meters (Grandcourt et al. 2005; Kuitert and Tonozuka 2001; Collette 2001; Pauly et al. 1996; Myers 1991). "Its distribution spans the Indo-Pacific waters with its range extending from the Red Sea and the eastern coast of Africa across Southeast Asia to East Asia, including China and Japan, and southward to Australian waters (Randall 1995). It primarily preys on teleost fishes, shifting to a predominantly piscivorous diet as it grows beyond 40 cm in length, although crustaceans such as shrimps are also significant in its diet (Bakhoun 2007).

The species Trypanorhynch parasites, which encyst in the musculature of fish, significantly impact both the health and marketability of affected species. These parasites cause fibrosis, tissue damage, and impaired health, with visible cysts in edible tissues reducing consumer appeal and market value (Shirakashi et al. 2012). Molecular tools like PCR have enabled precise identification of Trypanorhynchs, aiding in tracking their prevalence and distribution in both wild and farmed populations (Palm et al. 2007). However, ecological and histopathological studies of this group remain scarce despite their frequent taxonomic documentation worldwide. The present study investigates the parasitism of larval Trypanorhyncha worms infecting *S. commerson* commercialized in the Mappila Bay harbor of Kannur district, Kerala. It provides comprehensive data on the prevalence of larval Trypanorhyncha, seasonal variation, specific sites of infection, and associated histological changes. Histopathological investigation helps to reveal the kind of deterioration in the intestinal layers of the fish due to parasitic infestation such as necrosis, shrinkage, rupture of mucosal layers. Effective aquaculture practices, such as those implemented in Hawaii and Japan, have demonstrated the potential to disrupt the complex life cycles of parasites, significantly reducing infection rates (Dunne et al. 2020; Kleisner et al. 2021). Histopathological insights into parasitic infections are crucial for mitigating their economic impact, ensuring the marketability of seafood, and supporting the sustainability of aquaculture practices (Dias et al. 2011; Shirakashi et al. 2012).

2. MATERIALS AND METHODS

S. commerson (Fig.1) samples were collected from Ayikkara, Mappila Bay fish landing centre on the Malabar Coast, India, (Lat. 11° 51'33" N, 75° 22'30" E), (Fig. 2) during the pre-summer, summer, monsoon and post monsoon period of two consecutive years, 2019-20 and 20-21. After collection, the abdomen was opened by making an incision along the ventral midline extending from the anal region towards the mouth. The external surfaces of the intestine and associated visceral organs were then examined (Roberts, 2012). The alimentary canal was separated and placed in a saline medium. The guts of the collected fish were cut open, and carefully inspected using a stereomicroscope (Magnus–stereo zoom). Recovered cestodes were carefully removed from the gut and treated with a saline solution. Completely flattened specimens were stored in 70% alcohol. Following fixation, the parasites were stained using alum carmine, passed through a graded alcohol series for dehydration, rendered transparent using xylene and mounted permanently in DPX for detailed morphological examination and identification (Humason, 1979).

For the histopathological study, infected and uninfected intestinal tissues were fixed using 5% formalin, and processed through standard histological method after Humason (1979). The treated tissues were infiltrated with paraffin wax at 60 °C, allowed to solidify, and sectioned using a microtome. (Bancroft and Gamble, 2008). The sections were stained with hematoxylin, counter-stained with eosin, and mounted with DPX (Menoret and Ivanov, 2012). Slides were observed under the microscope, and photographs were taken by

using Invitrogen EVOS M5000 microscope (Thermo Fisher Scientific, MA, USA) at various magnifications, to compare the samples with the control and observe histopathological changes.

Fig. 1 Fish specimen, *Scomberomorus commerson* collected from Aykkara Fishing harbor, Kannur, Kerala



Fig. 2 Map showing study site, Aykkara Fishing Harbor, Kannur district, Kerala



Host fish were identified and their nomenclature verified using the FAO Species Identification Guide for Fishery Purposes (Collette, 2001), taxonomic position confirmed by Fish Base (Froese and Pauly 2021); and parasites were identified genus level, as the member of Order Trypanorhyncha Diesing, 1863, (Campbell and Beveridge (1994)). The prevalence is defined “as the number of hosts infected with one or more individuals of a particular parasite species divided by the total number of hosts investigated for that parasite species. It is often stated as a percentage when used descriptively and as a proportion when incorporated into mathematical models” (Bush, 1997).

Parasitic indices like prevalence, intensity, total number of parasites infested on a fish were observed for all examined hosts. The prevalence, mean intensities, median intensities, mean abundances and mean crowding were analyzed statistically using the software Quantitative Parasitology (QP 30). Significant variations in seasonal wise infestation and distribution of infested parasites were done by Kruskal-Wallis test of Past

Software version 4.03 (Hammer et al. 2001).

3. RESULTS:

3.1. Morphological analysis of the intestines of *S. commerson*.

The morphological examination of the intestines of *S. commerson* revealed the presence of encysted cestode larvae (plerocercoids) of the order Trypanorhyncha. Out of 61 fish examined, 52 were infected, with approximately 279 plerocercoids recovered from the intestinal region, resulting in a prevalence rate of 85.2% during the study period. Infestation was high in monsoon (September- November) (100%) and post monsoon period (June-August) (95%), but rate of infestation decreased during pre-summer (Dec-Feb) (76.9 %) and summer months (March - May) (50%) (Table 1 and fig. 3). There is a significant difference in the infestation rate and abundance of parasites among different seasons of the collection period showed (Kruskal-Wallis test $H(\chi^2): 17.18, p(\text{same}): 0.01193$ and $H(\chi^2): 17.39, p(\text{same}): 0.01365$ respectively).

Infected intestines having plerocercoids in the submucosal layer of the intestine wall, stomach did not infest by this cyst (fig. 4). Body of the encysted larvae consisted of a scolex and an appendix (AP). The scolex was differentiated into distinct regions, namely the pars bothridialis (PBO), pars vaginalis (PV), and pars bulbosa (PB) (Beveridge and Campbell, 2007)(Fig. 5). The larval cestodes were enclosed in white colored cyst (fig. 6). The recovered larvae had two bothria along with a tentacular apparatus bearing four retractable, hook-armed tentacles, indicating their affinity to the genus *Pintneriella* (de Sale Ribeiro et al. 2021).

Table 1 Statistics of plerocercod larvae infestation in the gut of *S. commerson* (Quantitative Parasitology - version 3.0).

Study period	No. of host fishes	Total no. of Infested	Prevalence	Mean intensity	Median Intensity	Mean abundance	Mean crowding
Pre-summer (Dec -Feb)	13	10	76.9%	6.7	6.0	5.15	7.18
Summer (March-May)	10	5	50.0%	11.60	10.0	5.80	12.59
Monsoon (Jun-August)	18	18	100.0%	3.72	3.0	3.72	3.93
Post monsoon (September-November)	20	19	95.0%	3.79	4.0	3.60	3.97
Total	61	52	85.2%	17.0	18.0	14.49	17.67

Fig. 3 Seasonal-wise variation in

occurrence of plerocercoid larvae infestation in the gut of *S. commerson*.

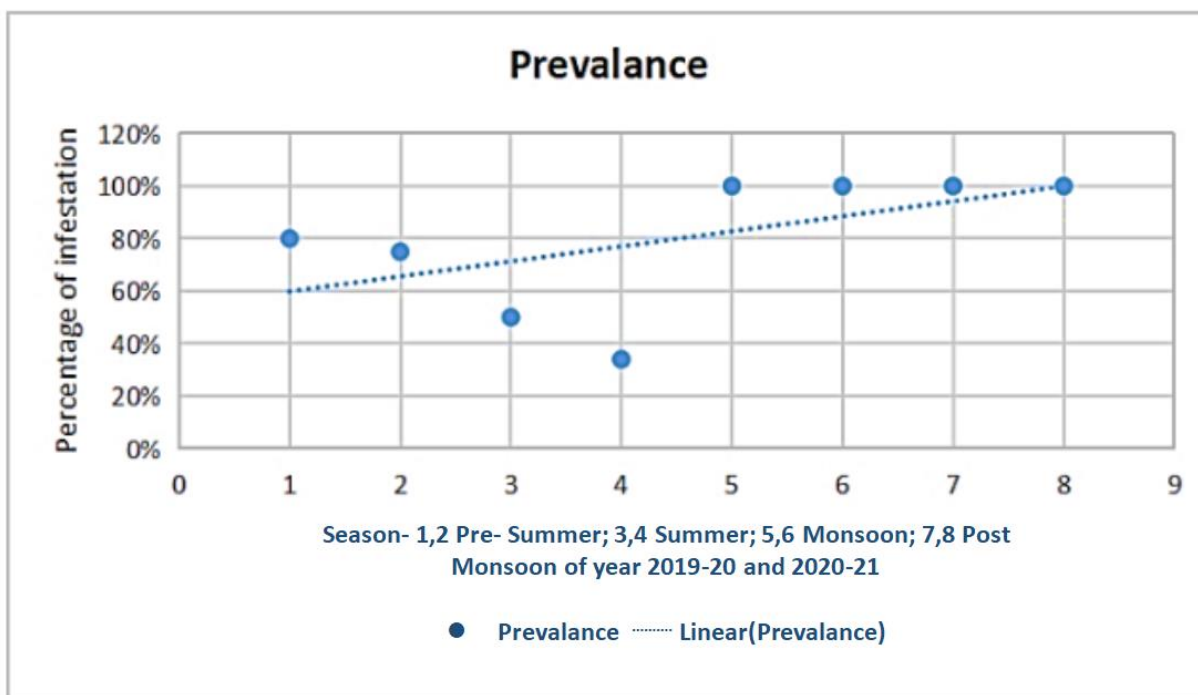


Fig. 4 Infected intestine shows encysted plerocercoid larvae in the submucosal layer



Fig. 5 Plerocercoid larva of the order Trypanorhyncha showing different body parts: Scolex (Pars bothridialis (PBO), Pars Vaginalis (PV), Pars bulbosa (PB)), and Appendix (AP).

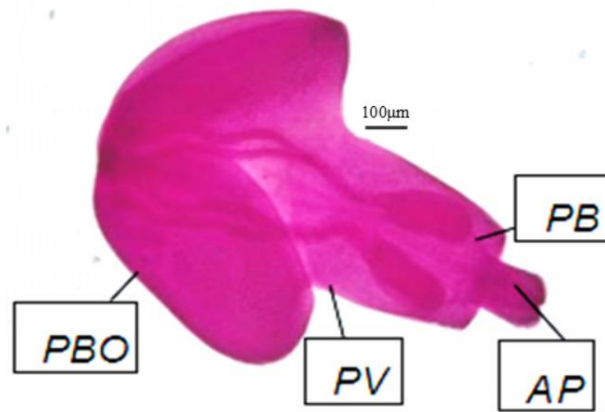


Fig. 6 Plerocercoid larva enclosed in white-coloured cyst. (



3.2. Effect of plerocercoid larvae of Trypanorhyncha parasites on histoarchitecture of the fish intestine

The presence of Trypanorhyncha parasites in fish intestines led to a variety of histopathological alterations. The observed changes were complex and multifaceted, encompassing several distinct pathological features. Infected intestines exhibited both acute and chronic inflammatory responses, characterized by the infiltration of neutrophils, lymphocytes, macrophages, and eosinophils. There was significant mechanical disruption to the epithelial lining, resulting in villous atrophy, erosion, and, in severe cases, necrosis, leading to the loss of tissue architecture. Chronic inflammation triggers the proliferation of fibroblast and building up of collagen, resulting in fibrosis and thickening of connective tissues (Fig. 7). Additionally, an elevated abundance of cells of intestinal mucosa, particularly goblet cells and enterocytes was observed, likely associated with epithelial damage and indicative of hyperplasia (Fig. 8). The blood vessels within the intestinal wall showed signs of congestion and haemorrhage, contributing to further tissue damage and inflammation. Finally, the function of intestinal glands was disrupted, resulting in altered mucus secretion, which impacted the protective mucus layer lining the intestine.

Photomicrographs of H&E-stained intestinal sections infected with plerocercoid larvae reveal hyperplastic, cystically dilated crypts encircled by radially oriented bundles of smooth muscle, in contrast to the normal architecture observed in uninfected tissue. The polyp surface exhibits fibro-inflammatory exudate along with granulation tissue, while the submucosal layer shows focal areas of necrosis and marked leukocyte infiltration, in contrast to the uninfected tissue (Fig. 7). Photomicrographs also show edema compared to the uninfected intestinal tissues and severely affected areas of mucosa were found compared to the uninfected

fishes (Fig. 8), inflammatory cell proliferation and infiltration were present compared to the uninfected fishes (Fig. 9). A dense aggregation of lymphoid cells was observed dispersed throughout the lamina propria of the posterior intestine. Histological pictogram showed high mucosal chronic inflammatory cells and villous blunting in the infected fishes compared to uninfected part. The presence of infected macrophages was detected in the submucosa to the corresponding control; macrophage aggregate surrounded by strong lymphocytic infiltration was also present. Fibrosis, thickening of connective tissue and vasodilation are present in the infected intestinal region (Fig. 10).

Fig. 7. Photomicrographs of H&E-stained intestinal tissue of *S. commerson* infected with plerocercoid larvae. Sections (B & D) display hyperplasia with “dilated crypts with cystic morphology” encircled by radially oriented smooth muscle bundles (arrows), in contrast to the normal morphology observed in uninfected tissues (A & C). The polyp surface in infected sections (B & F) shows fibro-inflammatory “exudate and granulation tissue”, whereas these features are absent in the corresponding control sections (A & E). The submucosal layer exhibits focal necrotic areas (F, arrow) along with leukocyte infiltration (F, black arrowhead).

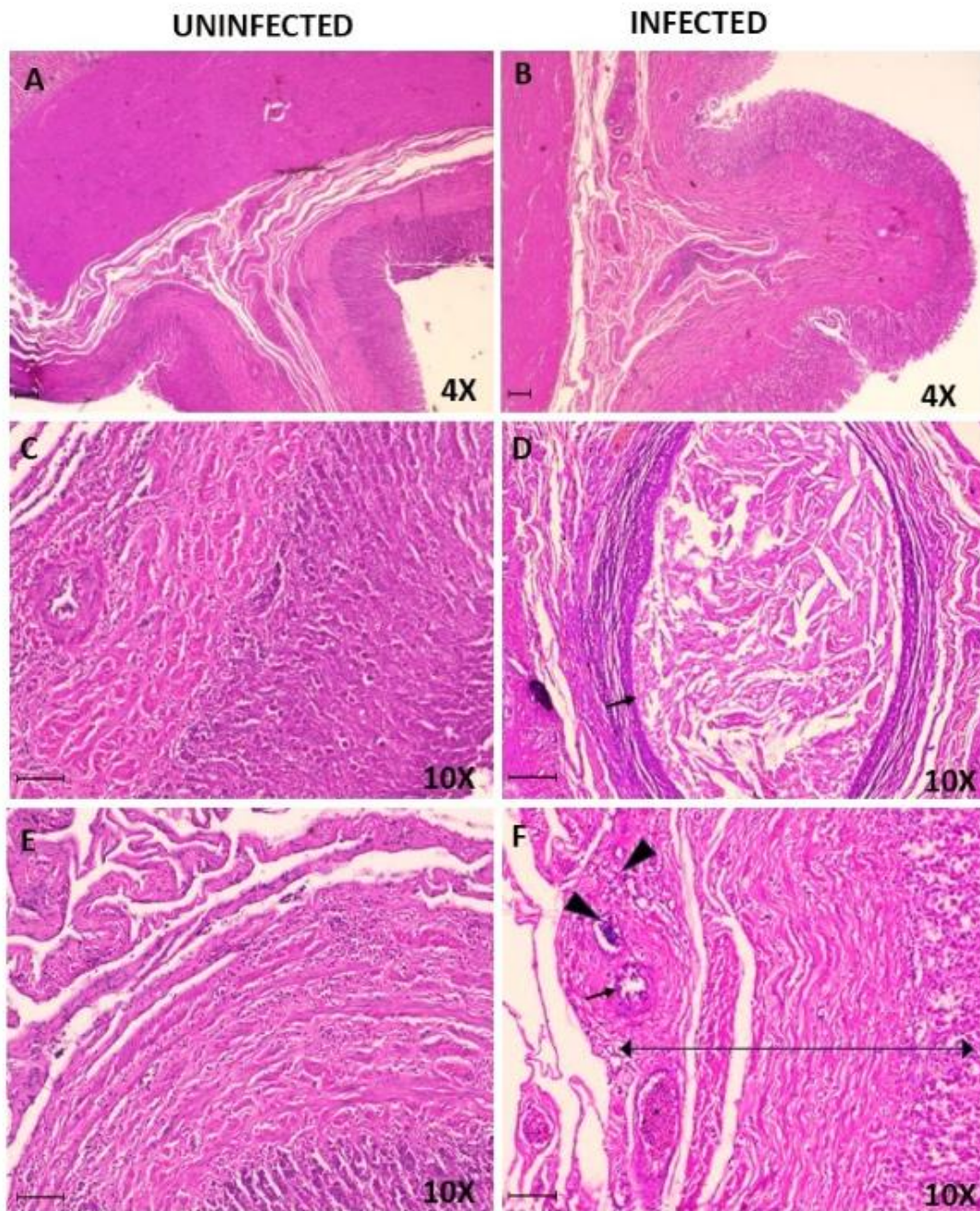


Fig. 8 Photomicrographs of H & E-stained Intestinal tissue of *S. commerson* infected by plerocercoid larvae. It shows edema (B, D, F) compared to the uninfected (A, C, E) and severely affected areas of

mucosa represented as ‘*’ (B & D) compared to the uninfected fishes (A & C).

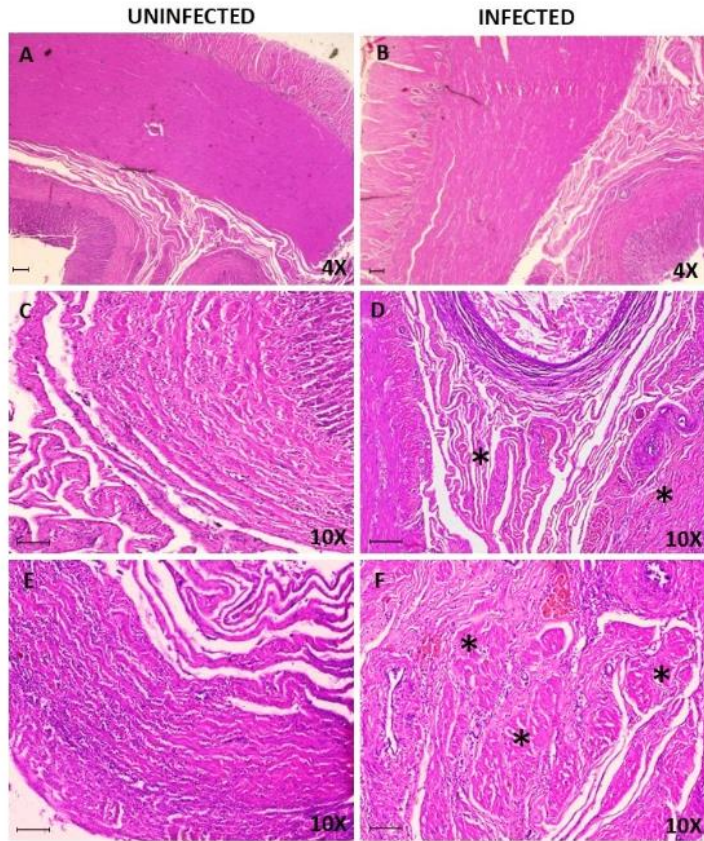


Fig. 9 Photomicrographs of H & E- stained Intestinal tissue of *S. commerson* infected by plerocercoid larvae. Inflammatory cell proliferation and infiltration were present in the (B, D, F) compared to the uninfected fishes (A, C, E). A high density of lymphoid cells was observed distributed throughout the lamina propria of the posterior intestine. indicated by ‘double headed arrow’ (D, F)

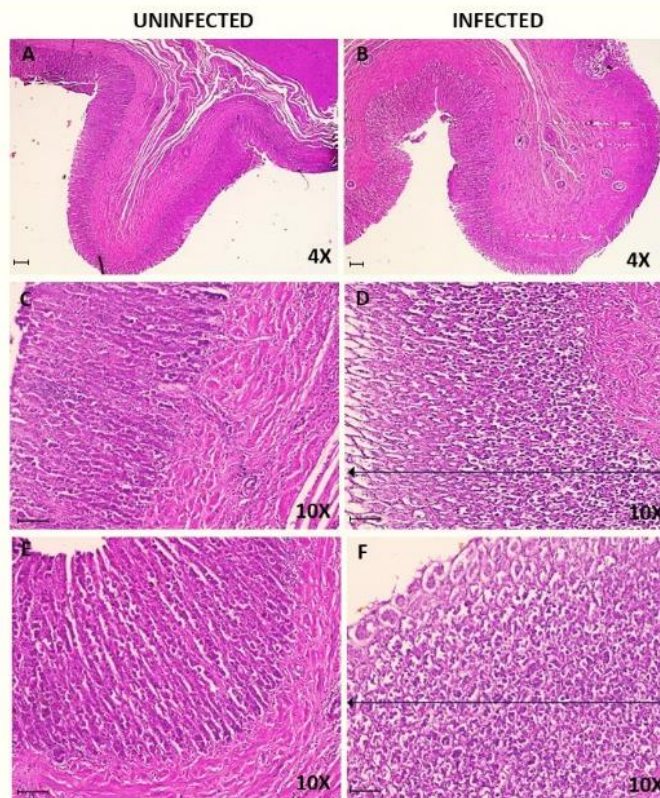
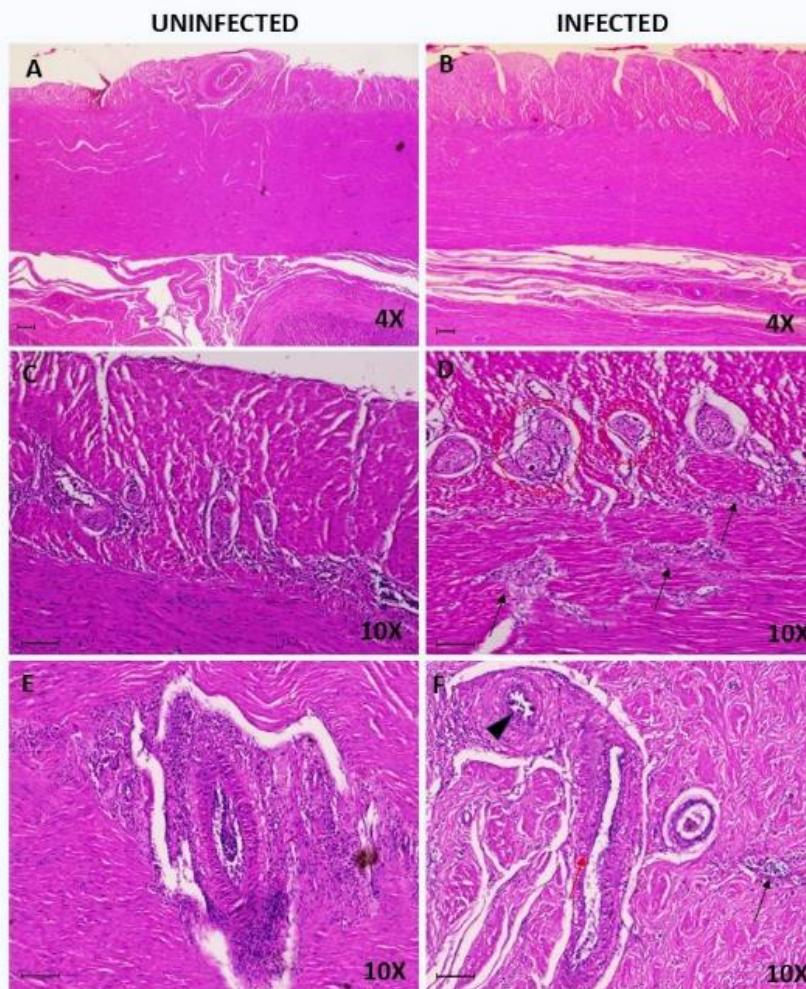


Fig. 10 Photomicrographs of H & E- stained Intestinal tissue of *S. commerson* infected by plerocercoid larvae. The infected samples exhibited increased infiltration of chronic inflammatory

cells in the mucosa along with blunting of the villi.(B) compared to uninfected part (A). The presence of infected macrophages was detected in the submucosa (B & D, indicated by the red circle) to the corresponding control (A & C); Macrophage aggregate surrounded by strong lymphocytic infiltration (D, black arrow) was also present. Fibrosis (F, black arrow), thickening of connective tissue (F, arrowhead), and vasodilation are present in the infected intestinal region (F, red arrow)



4. DISCUSSION

In a study by de Sale Ribeiro et al. (2021), larval tapeworms recovered from *Epinephelus marginatus*, wild-caught Dusky Grouper in the Canary Islands (Atlantic Ocean) were assigned to the genus *Pintneriella* (Yamaguti, 1934) of the family Rhopalothylicidae, based on their morphological features. Adult Trypanorhynch, which feature a scolex carrying 2 or 4 bothria and a tentaculam equipment with 4 retractable tentacles armed with many hooks, adapt for firm attachment to the tissues of the definitive host (Palm et al. 2009; Mehlhorn 2016). Four species are included within the genus *Pintneriella*: *P. musclicola*, *P. gymnorhynchoides*, *P. pagelli*, and *P. maccallumi*. Adult representatives of this genus survive in the intestinal tract of the final host, from where they release coracidial larvae into the surrounding marine environment (Palm, 2004). Those larvae are taken up by a primary intermediate host, where they develop into proceroid stages. Sooner or later, they may be ingested by the secondary intermediate host, penetrate via the intestine wall, encyst in the viscera or muscles, and mature right to plerocercus (Roberts 2012; Tamaru et al. 2016). Palm et al. (1994) reported nine trypanorhynchid species based on examinations of a diverse fish ranges of Gulf of Guinea. They suggested life cycle with four-hosts for these species, of which two are teleost hosts: small clupeids and large predatory fish and are obligatory for the completion of cycle. In a related study, de Sale Ribeiro (2021) found that 96% of observed Dusky Groupers from the Canary Islands had trypanorhynch plerocerci infestation. This examination also extended to the damage caused by these cestode parasites, showing many cysts and nodules filled with larvae in the abdominal cavity. These were found in areas with a lot of fibrosis and led to visceral adhesions. Trypanorhynch parasites were reported from king mackerel (*Scomberomorus cavalla*) collected in Miami, USA (Ward, 1954). Similarly, a study conducted in Niterói and Rio de Janeiro, Brazil, revealed that 53% of the examined *S. cavalla* individuals were infected with Trypanorhyncha metacestodes (Dias et al., 2011). The infection of fish intestines by plerocercoid larvae of

Trypanorhyncha parasites induced a variety of histopathological changes that collectively impacted the health and functionality of the affected organs. These changes included acute and chronic inflammation, epithelial disruption, fibrosis, hyperplasia, vascular alterations, and glandular modifications. These pathological responses were documented in several studies in other species of fish, providing insight into the mechanisms and consequences of parasitic infections in fish. The initial response to Trypanorhyncha infection was characterized by acute inflammation, primarily involving the infiltration of neutrophils as part of the innate immune response (Woo 1995). As the infection persisted a chronic inflammatory response developed, dominated by lymphocytes, macrophages, and eosinophils, which were crucial for adaptive immunity and combating parasitic infections (Roberts and Janovy 2009). Chronic inflammation often leads to granuloma formation, where macrophages organize into granulomas to encapsulate and contain the parasites (Ferguson 2006). This granulomatous response was a hallmark of prolonged parasitic infections and reflected the host's attempt to isolate the persistent irritants.

The physical presence and activity of Trypanorhyncha larvae caused significant mechanical disruption to the epithelial lining of the intestines. This disruption resulted in villous atrophy, where the normally finger-like projections of the mucosa became flattened or eroded, impairing the absorptive surface area (Williams and Jones 1994). In severe infections, the damage extends to necrosis, where epithelial cells undergo apoptosis, leading to the loss of tissue architecture and function (Roberts 2012). Necrotic areas were characterized by zones of dead cells, which further compromised the integrity and absorptive capabilities of the intestinal lining. Chronic inflammation stimulated the proliferation of fibroblasts and the deposition of collagen, leading to fibrosis. This process resulted in the thickening and scarring of connective tissue within the intestine (Ferguson 2006). Fibrosis represented a maladaptive response to chronic injury, as the excessive collagen deposition disrupted the normal tissue architecture and elasticity, impairing intestinal motility and function. The fibrotic changes could lead to a permanent loss of function in the affected areas, contributing to long-term health issues in the infected fish.

In response to epithelial damage, there was often a compensatory increase in the number of mucosal cells, known as hyperplasia. This increase particularly affected goblet cells and enterocytes, as the intestine attempted to regenerate its damaged lining and maintain its barrier function (Roberts and Janovy 2009). Hyperplasia could lead to the thickening of the mucosal layer, which might be a protective response but could also alter normal tissue function and nutrient absorption. The congestion and haemorrhage were observed in the intestines of the fish. Trypanorhyncha larvae damage the blood vessels within the intestinal wall, leading to vascular changes such as congestion and haemorrhage. Congestion refers to the excessive accumulation of blood within blood vessels, whereas hemorrhage is the escape of blood from vessels into adjacent tissues (Williams and Jones, 1994). These vascular alterations caused localized blood extravasation, visible as dark red spots or patches, and contributed to further tissue damage and inflammation.

The presence of Trypanorhyncha parasites and the associated inflammatory response disrupted the normal function of intestinal glands, including those responsible for mucus production. This disruption resulted in either increased or decreased mucus secretion, impacting the protective mucus layer that lined the intestine (Roberts 2012). Altered mucus production affected the overall intestinal environment, influencing factors such as pathogen resistance, nutrient absorption, and gut motility. In *S. commerson* infected with parasitic cysts, several pathological changes are noticeable, the intestinal layer becomes pale, and the lumen fills with mucus. The severity of tissue damage correlates with the intensity of infection. Similar studies by Ibrahim (2000), Maftuch et al. (2017), and de Sale Ribeiro et al. (2021) show that helminth parasites cause extensive damage at attachment sites, disrupting the intestine's absorption efficiency.

Plerocercus larvae of trypanorhynchid cestodes were found infesting the intestinal walls of the commercially important marine fish, *S. commerson*, on the Malabar Coast of India. These parasites can adversely affect fish health, growth rates, reproductive success, and market value, impacting the economic viability of fisheries. However, the present study reveals that trypanorhynchid parasites may not significantly impact the marketability of *S. commerson* due to the fish retaining its appealing appearance; they do notably affect the growth and survival of kingfish. Some reports did indicate potential allergic reactions in humans consuming raw or semi-raw fish meat infested with Trypanorhyncha species (Gomez-Morales et al. 2008). Histopathological analysis revealed significant epithelial damage, mucosal alterations, and structural changes in the intestinal layers due to Trypanorhyncha infection. These findings underscore the importance of understanding the pathological responses for effective disease diagnosis, management, and control strategies in aquaculture and fisheries, ultimately aiming to sustain fishery yields and industry viability. By preventing exposure to infected wild organisms, aquaculture ensures healthier, parasite-free fish, enhancing the industry's economic viability and contributing to conservation efforts (Yoshinaga et al. 2020).

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6. HIGHLIGHTS

- First report of Trypanorhyncha cestodes in *Scomberomorus commerson* from southwest India.
- High infection prevalence (85.2%) with plerocercoid larvae encysted in *S. commerson* intestine.
- Histopathology shows epithelial hyperplasia/atrophy driven by host immunity, not parasite feeding.
- Inflammatory responses around cysts impair nutrient absorption in host tissues.
- Emphasizes the need for monitoring parasitic impacts on marine fisheries sustainability.