



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

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<p>Article History</p> <p>Received: Accepted: Published:</p> <p>CC License CC-BY-NC-SA 4.0</p>	<p>Abstract</p> <p>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, <i>Scomberomorus commerson</i>. 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 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 <i>S. commerson</i> 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..</p> <p>Keywords: Trypanorhyncha, Cestoda, Plerocercoid, Histopathology, <i>Scomberomorus commerson</i>.</p>
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1. INTRODUCTION

Trypanorhynch cestodes are common parasites of marine fish, with a complex life cycle involving three hosts. The 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 procercooids. These procercooids are then consumed by teleost fish or invertebrates, the second intermediate hosts, where they penetrate the gut wall, encyst in the viscera or musculature, and mature into plerocercoids before reaching the final host (Overstreet, 1978; Palm 2000; Alvarez et al. 2006; Haseli et al. 2010; Beveridge et al. 2014; Alves et al. 2017). These parasites demonstrate lower host specificity during their larval stages, enabling widespread distribution from brackish waters to deep-sea environments, with notable diversity in coastal tropical waters of the Indo-Australian region (Palm and Cairn 2008; Palm et al. 2009). Their presence in economically significant fish species, such as the Narrow-barred Spanish

mackerel, *Scomberomorus commerson*, underscores their ecological and economic importance. Building on their intricate life cycle, members of the cestode order Trypanorhyncha Diesing (1863), 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, along with a tentacular apparatus comprising four retractile tentacles adorned with hooks and connected to four bulbs, serves as a key diagnostic feature (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; Beveridge and Campbell 2007; Friggens and Duszynski 2005; Beveridge and Justine 2006; Beveridge and Justine 2007; Campbell and Beveridge 2006; 2007; and Palm et al. 2009). Extensive taxonomic studies have been conducted in various regions: the Gulf of California, Java, Borneo, Australia, New Caledonia, Hawaii, Japan, Iran, Indonesian coastal waters, the Egyptian Red Sea, the Gulf of Guinea, the Arabian Gulf, and the Red Sea in Yemen (Overstreet 1977; Palm et al. 1994; Palm 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; Ibrahim 2000; Al-Zubaidy and Mhaisen 2021). However, the present study marks the first report of Trypanorhyncha infestation in the commercially important host fish *Scomberomorus* sp. from the southwest coast of India, highlighting the possibility for further research into their distribution and host-parasite interactions in this region.

The Narrow-barred Spanish mackerel, *Scomberomorus commerson* (Lacepede 1800) of the Scombridae family, is a highly valued edible fish with significant market importance along the Malabar Coast. This species is marketed fresh, dried, or salted, and any threat to its population can critically impact the local fishing industry. *S. commerson* is an epipelagic and oceanodromous fish (Riede 2004), inhabiting areas from the continental shelf edge to shallow coastal waters at depths up to 200 meters (Myers 1991; Pauly et al. 1996; Kuitert and Tonozuka 2001; Collette 2001). Its distribution spans the Indo-Pacific, from the Red Sea and South Africa to Southeast Asia, extending north to China and Japan, and south to Australia (Randall 1995). The species primarily preys on teleost fishes, shifting to a predominantly piscivorous diet as it grows beyond 40 cm in length, though shrimps are also significant in its diet (Bakhoun 2007).

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

Fresh specimens of *Scomberomorus commerson* (Fig.1) were collected from Ayikkara (Lat. 11° 51'33" N, 75° 22'30" E), one of the major fish landing centers on the Malabar Coast, India, (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 cut open along the mid ventral line from the anal region to the mouth. The surface of the intestines and visceral organs was examined. 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. After fixation, the parasites were stained using alum carmine, dehydrated in ascending grades of alcohol (30%, 50%, 70%,

90%, and 100%), cleared in xylene, and mounted in DPX for further identification and detailed examination.

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



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



Host fish identification, nomenclature, and taxonomy were conducted according to the FAO Species Identification Guide for Fishery Purposes (Collette 2001) and Fish Base (Froese and Pauly 2021); and parasites were identified based on the classification by Campbell and Beveridge (1994) as belonging to the Order Trypanorhyncha Diesing, 1863. 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.

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

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variations in seasonal wise infestation and distribution of infested parasites were analysed using Kruskal-Wallis test by Past Software version 4.03 (Hammer et al. 2001).

For the histopathological study, infected and uninfected intestinal tissues were fixed in 5% formalin, dehydrated in ascending grades of alcohol, and cleared. They were then embedded in paraffin wax at 60°C, solidified, and sectioned using a microtome. The sections were stained with hematoxylin, counter-stained with eosin, and mounted with DPX. Slides were observed under a microscope, and photographs were taken using an Invitrogen EVOS M5000 microscope (Thermo Fisher Scientific, MA, USA) at various magnifications, to compare the samples with the control and observe histopathological changes.

3. RESULTS:

3.1. The 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 (chi2): 17.18, p (same):0.01193 and H(chi2):17.39, p (same): 0.01365 respectively). Infected intestine having plerocercoids in the submucosal layer of the intestine wall, stomach did not infest by this cyst (fig. 4). These encysted larvae have different body parts: scolex with Pars bothridialis (PBO), Pars Vaginalis (PV), Pars bulbosa (PB) and Appendix (AP) (fig. 5). The larval cestodes were enclosed in white colored cyst (fig. 6). The morphological features of the recovered larvae contain two bothria and a tentacular apparatus with four retractable tentacles armed with numerous hooks, suggesting this species belongs to the Pintneriella genus.

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*.

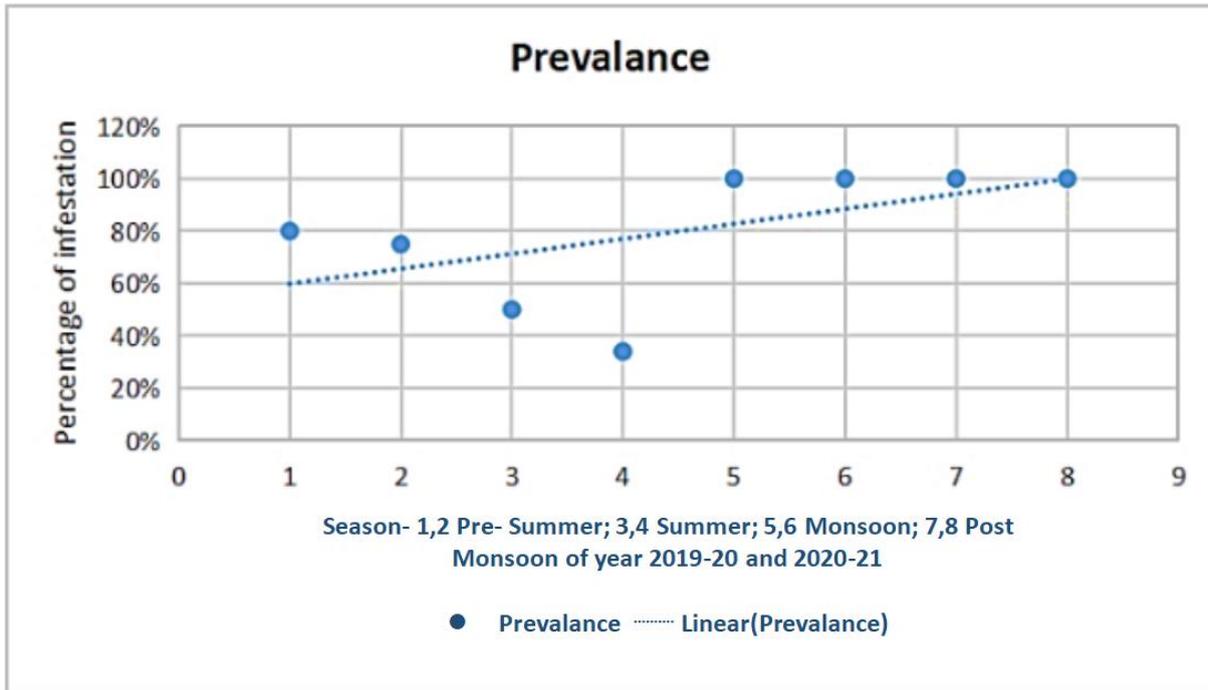


Fig. 4 Infected intestine shows encysted plerocercoid larvae in the submucosal layer



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

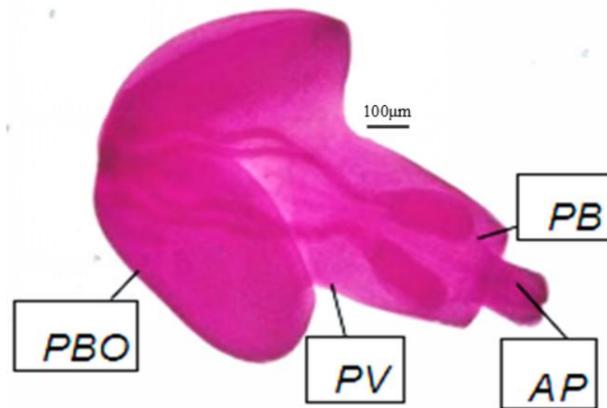


Fig. 6 Plerocercoid larva enclosed in white-coloured cyst. The body of encysted larvae is divided into scolex with pars bothridialis, pars vaginalis, and pars bulbosa and appendix.



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 stimulated fibroblast proliferation and collagen deposition, causing fibrosis and thickening of connective tissue (Fig. 7). Additionally, there was an increase in the number of mucosal cells, particularly goblet cells, and enterocytes, in response to epithelial damage, 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 tissue infected by plerocercoid larvae shows hyperplastic and

cystically dilated crypts surrounded by radially arranged smooth muscle bundles, corresponding to the uninfected section. The surface of the polyp shows fibro-inflammatory exudate and granulation tissue compared to the uninfected part and submucosa layer with regions of necrosis and leukocyte infiltration (Fig. 7). Photomicrographs also shows 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). High concentration of lymphoid cells was found scattered in 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 by plerocercoid larvae. It shows Hyperplastic and cystically dilated crypts surrounded by radially arranged smooth muscle bundles (B & D, arrow), corresponding to the uninfected section (A & C). The surface of the polyp shows fibro-inflammatory exudate and granulation tissue (B & F) compared to the uninfected part (A & E). Submucosa layer with regions of necrosis (F, arrow) and leukocyte infiltration (F, black arrowhead).

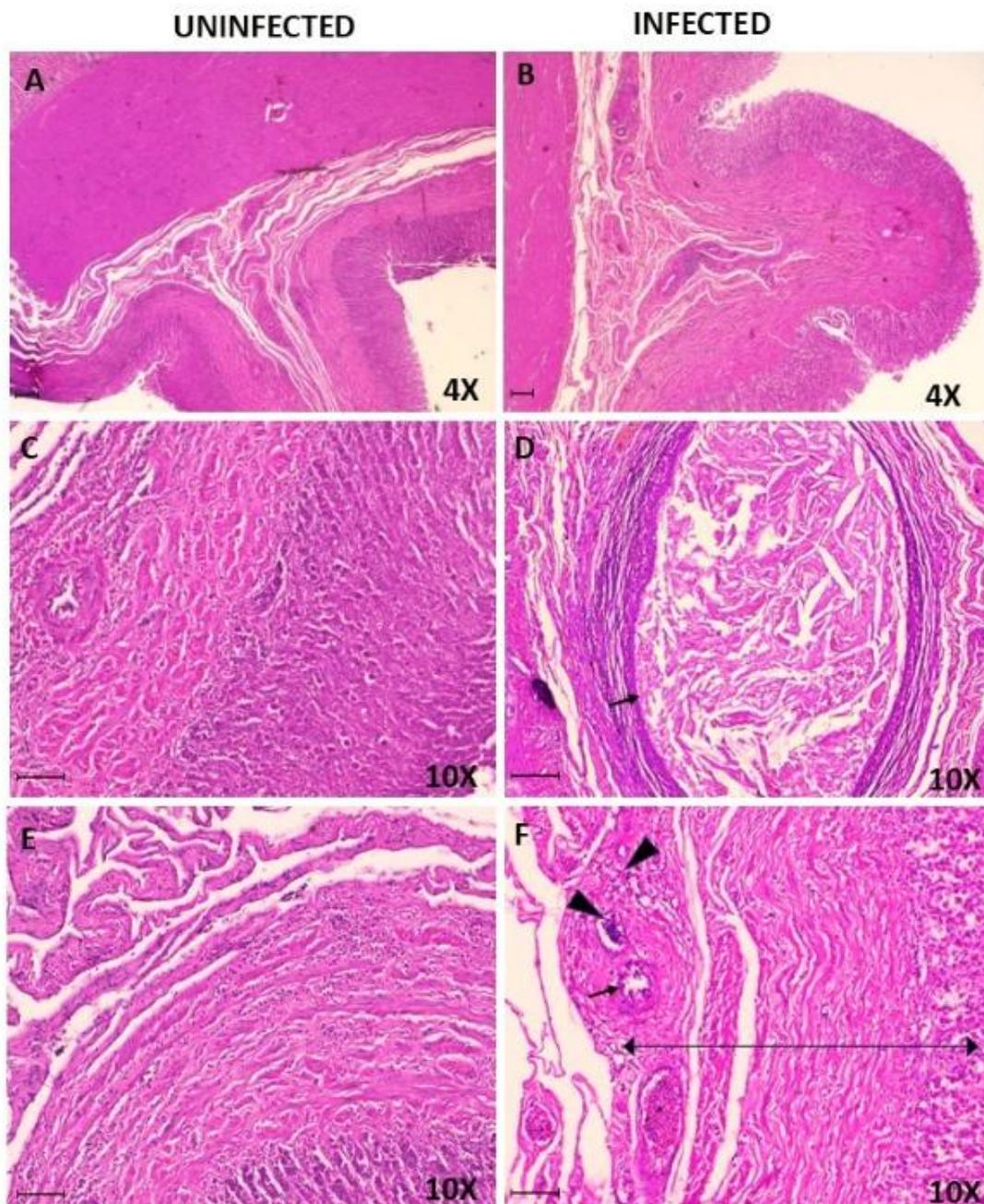


Fig. 8 Photomicrographs of H & E-stained Intestinal tissue of *S. commerson* infected by plerocercoid larvae. It shows edema (B, D, F) compared to the uninfected (A, C, E) and severely affected areas of mucosa represented as ‘*’ (B & D) compared to the uninfected fishes (A & C).

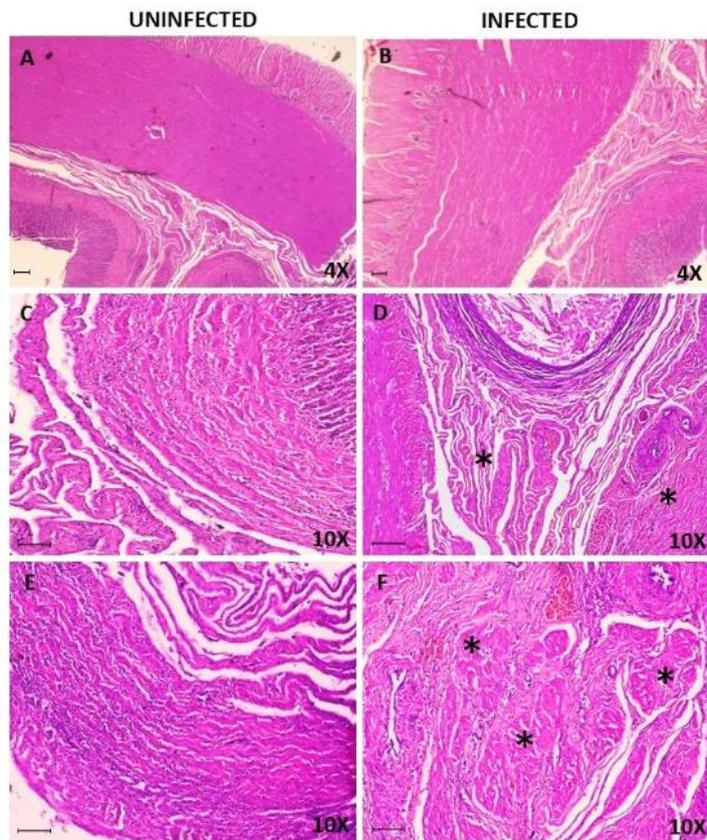


Fig. 9 Photomicrographs of H & E- stained Intestinal tissue of *S. commerson* infected by plerocercoid larvae. Inflammatory cell proliferation and infiltration were present in the (B, D, F) compared to the uninfected fishes (A, C, E). High concentration of lymphoid cells was found scattered in the lamina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

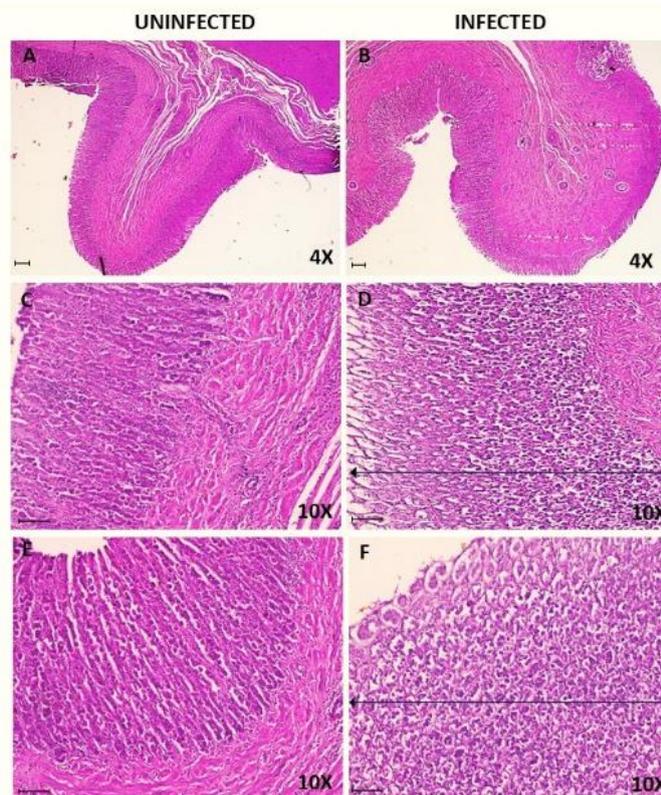
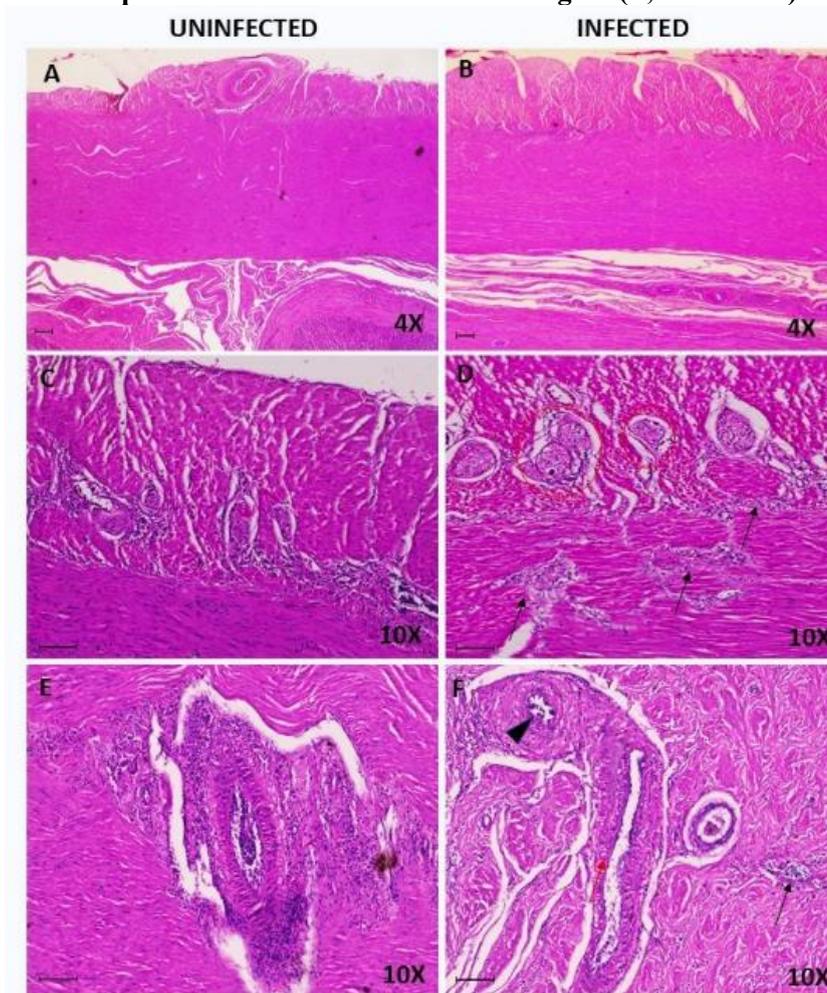


Fig. 10 Photomicrographs of H & E- stained Intestinal tissue of *S. commerson* infected by plerocercoid larvae showing a mucosal chronic inflammatory cell increase and villous blunting in the infected samples (B) compared to uninfected part (A). The presence of infected macrophages was detected in the 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), tapeworms identified as belonging to the genus *Pintneriella* (Yamaguti 1934), within the family Rhopalothylacidae, were found in wild-caught Dusky Groupers (*Epinephelus marginatus*) in the Canary Islands, Atlantic Ocean. This identification was based on the larval morphological characteristics. Adult trypanorhynch, which feature a scolex with two or four bothria and a tentacular apparatus with four retractable tentacles armed with numerous hooks, adapt to the attachment site in the final host (Palm et al. 2009; Mehlhorn 2016). Palm (2004) identified four species within *Pintneriella*: *P. musculicola*, *P. gymnorhynchoides*, *P. pagelli*, and *P. maccallumi*. The adult stage of *Pintneriella* species inhabits the gastrointestinal tract of the definitive host, where it releases free-swimming coracidium larvae into the marine environment. These larvae are ingested by a first intermediate host and converted into proceroids. Subsequently, they are ingested by a second intermediate host, penetrate through the gut wall, encyst in the viscera or musculature, and mature into a plerocercus (Roberts 2012; Tamaru et al. 2016).

In a study by Palm et al. (1994), nine trypanorhynchid species were identified by examining a wide spectrum of fish hosts in the Gulf of Guinea for infestation. They proposed a possible four-host life cycle for these species, involving two obligatory teleost host fish species: small clupeids and large predatory fish. In a related study, de Sale Ribeiro (2021) determined that the prevalence of trypanorhynch plerocerci was 96% in Dusky Groupers from the Canary Islands. This study also explored the pathological changes produced by Trypanorhyncha, revealing numerous larvae-filled cysts and nodules in the abdominal cavity,

embedded in abundant fibrosis and producing visceral adhesions. Trypanorhynchids were recovered from specimens of king mackerel, *Scomberomorus cavalla*, in Miami, USA (Ward 1954). 53% of observed *S. cavalla* specimens from Niterói and Rio de Janeiro, Brazil, were also found to be parasitized 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 damaged the blood vessels within the intestinal wall, leading to vascular changes such as congestion and haemorrhage. Congestion is characterized by the accumulation of blood in the vessels, while hemorrhage involves the leakage of blood into the surrounding 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 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, Available online at: <https://jazindia.com>

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 fish intestine.
- Histopathology shows epithelial hyperplasia/atrophy driven by host immunity, not parasite feeding.
- Inflammatory responses around cysts impair nutrient absorption in host tissues.
- Emphasizes need for monitoring parasitic impacts on marine fisheries sustainability.