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New Insights on the Effects of Monogenean Gill Parasites on Naturally Infested *Scoberomorus commerson*: Host Response, Electron Microscopy, and Histopathological Studies

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Abstract

Fish are frequently regarded as the most significant source of income due to their high economic value. The infection of monogeneans is the main cause of fish anemia leading to high mortalities. Thus, this study aimed to detect monogeneans parasites in *Scoberomorus commerson* and investigate the histopathological changes related to its natural infections. Furthermore, to research the potential parasite-host interactions. One hundred marine fishes (*S. commerson*) from the Ezbet-El Borg area, Damietta province, Egypt were examined from August 2022 to January 2023 for the presence of monogenean gill parasites. 38 fish were infected with monogenean species: *Pricea multae* 32% and *Gotocotyla acanthura* 19%. The structure of *P. multae* and *G. acanthura* were explained by using light and scanning electron microscope (SEM), the infected fish have no pathognomonic lesions, but for a few cases showed excessive mucus secretion. The pathological and ultrastructural results revealed massive branchial damage with partial to complete lamellar fusion and lamellar desquamation with complete loss of secondary lamellae and lymphocytic infiltration, the host response includes the appearance of numerous ruffled lymphocytes and mucous globules at the site of parasitic attachment.

Keywords: Monogenea; *Scoberomorus commerson;* Electron microscope; Histopathological alterations; Host response

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1. Introduction

Fish are frequently regarded as the most significant source of income due to their high economic value (Mehanna, 2022). Many kinds of highly valuable marine fish migrate, especially those of the Scombridae family. *Scomberomorus commerson* (Scombridae) is a predatory marine fish that is among the most expensive and high-quality fish, due to its nutrient-rich proteins (Claereboudt et al., 2005; Rajesh et al., 2017; Thai et al., 2021)

The narrow-barred Spanish mackerel (*S. Commerson*, Lacépède, 1800) concerns the ICCAT Convention as distributed through the most of Pacific Ocean and Indian Ocean including the Persian Gulf and the Red Sea, according to Di Natale et al. (2009). *S. Commerson* migrate to the Mediterranean Sea through the Suez Canal (Di Natale et al., 2020). The important and highly valuable epipelagic fish *S. Commerson* (narrow-barred Spanish mackerel) has been caught in Egypt for many years using purse seines (PS), gillnets (GLLL), and rod-and-reels (RR or SPOR) (Dimech et al., 2012). Biology knowledge is crucial for prevention since environmental parasites pose a greater threat to this fish (Nack et al., 2018).

Monogenea is a parasitic flatworm that has been found in fish throughout the world, but it has just recently been discovered in marine fish (Morsy et al., 2018). It attaches to its host through a posterior haptor (opisthaptor) that has suckers or clamps (Hayward, 2005). Scanning electron microscopy provides clear resolution and magnification to highlight specific organs of attachment in distinct types of helminths and taxonomic characteristics (Tadros et al., 2014; Yoon et al., 2013). The infection of monogeneans is the main cause of anemia and possible host mortality (Rigos et al., 2021; Woo and Bruno, 2011). Few research has been conducted in Egypt on the pathological alterations caused by monogenean parasites to the gills of marine fish hosts (EL-Naggar et al., 2019). In the host, changes such as physiological (cell proliferation, immunosuppression, altered growth, and negative behavioral responses) or mechanical damage (fusion of gill lamellae, epithelial desquamation, hypertrophy, and tissue replacement) are indicators of the parasites' negative effects (Igeh and Avenant-Oldewage, 2020). Due to the implantation of their hooks, monogenea also cause hemorrhages, necrosis, and desquamation of lamellar epithelium. Leukocyte infiltration and erosion were visible in the gills of the monogenean-infected *S. commerson* (Ali and Ismail, 2022). This study's objectives are to detect monogeneans parasites in *S. commerson* and investigate the histopathological changes related to its natural infections. Furthermore, to research potential parasite-host interactions.

2. Materials and methods:

2.1. Fish samples

A total of 100 freshly caught *S. Commerson* (narrow-barred Spanish mackerel) were collected alive or freshly dead from fishermen in the market of Ezbet- El Borg area, Damietta province, Egypt, during the period from August 2022 to January 2023, collected samples of fish with different body lengths (25-50cm) and weights (250-960gm) were transported on thick ice polyethylene bags to the laboratory of Animal Health Research

Institute, Mansoura Branch for examination. The experimental procedures were approved by the Institutional Aquatic Animal Care and Use Committee (IAACUC), Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafrelsheikh, Egypt. Approval Code: IAACUC-KSU-013-2023.

2.2. Parasitological examinations

2.2.1. Clinical Examinations

Examination of fish samples for detection of external parasites or any abnormalities (Eissa, 2016).

2.2.2. Light microscopic examinations

The gills were dissected and placed in Petri dishes with some distilled water and examined for the presence of monogeneans using a dissecting binocular microscope. The detected monogeneans were collected by a long-tipped Pasteur pipette to another petri dish containing 0.9% NaCl to remove excess mucous then fixed in 10% formalin, and finally washed with distilled water to remove excess fixative. Acetic acid alum carmine was used for staining for 15-30 minutes, dehydration by ascending grades of ethyl alcohol (70%-100%), cleared in clove oil, and mounted in Canada balsam (Carleton et al., 1967).

2.2.3. Scanning electron microscopy (SEM)

The isolated monogeneans specimens were fixed in 4% aqueous glutaraldehyde solution (4ºC for 48 Hrs.). Then they were washed thoroughly with cacodylate buffer and post-fixed for 4 hrs. with aqueous osmium tetroxide (Os O4) and dehydrated through alcohol. Later, they were dried in Tousimis Autosamdri -815 Coatar, E300 critical point drying apparatus using liquid CO_2, according to Bayoumy et al (2006) the specimens were whole–mounted on an aluminum stub and fixed by a double-phase sticker. The specimens were coated with gold-palladium in a Sputter Coating Evaporator unit (S.P.I. Model-Sputter Carbon/ Gold Coater) and then examined using a JEOLJEM-2100 scanning microscope operating at 20 Kev. All preparations were done at the EM Unit, Mansoura University, Egypt.

2.3. Histopathological examination

The affected parts of the gills were dissected and fixed in 10% neutral buffered formalin, then dehydrated in ascending grades of alcohol, cleared in xylol, and then embedded in paraffin wax. Five-micron sections were prepared and then routinely stained with Hematoxylin and Eosin (H&E) according to Suvarna et al. (2018), then examined under the light microscope (Olympus CX 31 microscope).

2.4. PAS staining

Sections were deparaffinized and hydrated in deionized water. The deparaffinized sections were soaked in Periodic Acid Solution for 5 minutes at room temperature and then Rinsed in several changes of distilled water. After that, the slides were placed in Schiff's Reagent for 15 minutes at room temperature and washed in running tap water for 5 minutes. The slides were Counterstained with Hematoxylin Solution then rinsed in running tap water and examined using the light microscope.

3. Results

Our study revealed that 38 out of 100 examined *S. commerson* were infected with monogenean gill parasites. The examined fish showed a higher infection rate with *P. multae* (32%) than *G. acanthura* (19%). No pathognomonic lesions in the naturally infected fish were noticed except in some cases suffered from excessive mucous secretions and a marbling appearance with the presence of parasites attached to the infected gills.

3.1. Morphological description of the detected parasites

The taxonomy of the recovered parasites is Family: *Gastrocotylidae*, Subfamily: *Priceinae*, Genus: *Pricea*, Species: *Pricea multae* (**Figure 1**). The worms were elongated smooth, and dorsoventrally flattened bodies. Total length is 4.2-5.5 mm, maximum width 1.5-1.8 mm at the level of ovary. The anterior end is rounded with a subterminal oral sucker. The pharynx is small, rounded, weekly muscula. The copulatory organ included 11-14 cirrus circle hooklets. The uterus is small, spindle-shaped containing a single spindle-shaped egg with a long filament at each pole.

The other parasite taxonomy is Family: Gotocotlyidae, Subfamily: Gotocotylinae, Genus: Gotocotyla, Species: *Gotocotyla acanthura* (**Figure 2**). The body is long, smooth, curving to the right, flattened dorso-ventrally with pointed anterior end. Total length is 8.2-10.0 mm including prohaptor, maximum width 1.7-1.9 mm in the middle region. The mouth was subterminal or ventral. The cirrus was observed to be everted from the genital atrium and armed with pectinate spines. The clamps were asymmetrical, numerous, and present at the anterior end of the opisthaptor with one pair of hamuli present at the end of the opisthaptor.

3.2. Histopathological results

The histopathological examination of infested gills revealed the presence of monogean parasites in interlamellar spaces with massive destruction of the lamellar epithelium (**Figure 3A**). The parasite had a smooth cuticle with a submuscular layer and a parenchymatous body and was admixed with necrotic lamellar epithelial debris (**Figure 3A**). Additionally, extensive lamellar desquamations and lamellar lifting with partial to complete lamellar fusion were also seen (**Figure 3B**). The gills were invaded with severe cellular infiltrations composed of numerous lymphocytes (**Figure 3B** and **C**). Other branchial sections showing pillar cell damage resulted in lamellar clubbing and desquamation beside lamellar tip hypertrophy. The desquamated secondary lamellar structure and the damaged primary lamellae were infiltrated with numerous lymphocytes, a few macrophages, and mucous globules (**Figure 3D**).

PAS staining results showed excessive dark pink mucoid patches and mucous cells either on the base of secondary lamellae or in interlamellar spaces surrounding the invaded parasites and admixed with numerous lymphocytes, macrophages, and desquamated lamellar epithelium (**Figure 4**).

3.3. Scanning electron microscopy results

The infected gills were examined using scanning electron microscopy to investigate the structure of the monogenean parasite and its interaction with the host**.** The result showed that *Pricea multae* had a ridged tegumental body with a microvillous-like projection around the preoral opening and genital atrium (**Figure 5A**). The haptor had preoral pits, preoral uniciliated sensilla, and an oral sucker without anchor hooks on the ventral surface of the anterior body part (**Figure 5B**). Posteriorly, compound clamps were detected as U-shaped Sclerites with marginal hamulus, inner lateral ribs, and median accessory sclerite (**Figure 5C**).

The structure of *Thoracocotyle indica* was also detected using SEM. The integumental body surface appeared transversal ridged with unciliate dome-shaped papillae (**Figure 5D**). The prohaptor showed a pro-oral opening and oral sucker with a pair of anchor hooks (**Figure 5E** and **F**). The opishaptor had two rows of clamps that had a median sclerite and 10 rib-like sclerites with a pair of humulus (**Figure 5D**). The interactions of this parasite with branchial tissue were also seen. Numerous ruffled lymphocytes were seen to invade the attached parasite and surround the desquamated lamellar filaments (**Figure 5G**). Furthermore, mucous production, lamellar fusion, and other cellular infiltrates are also observed (**Figure 5H** and **I**).

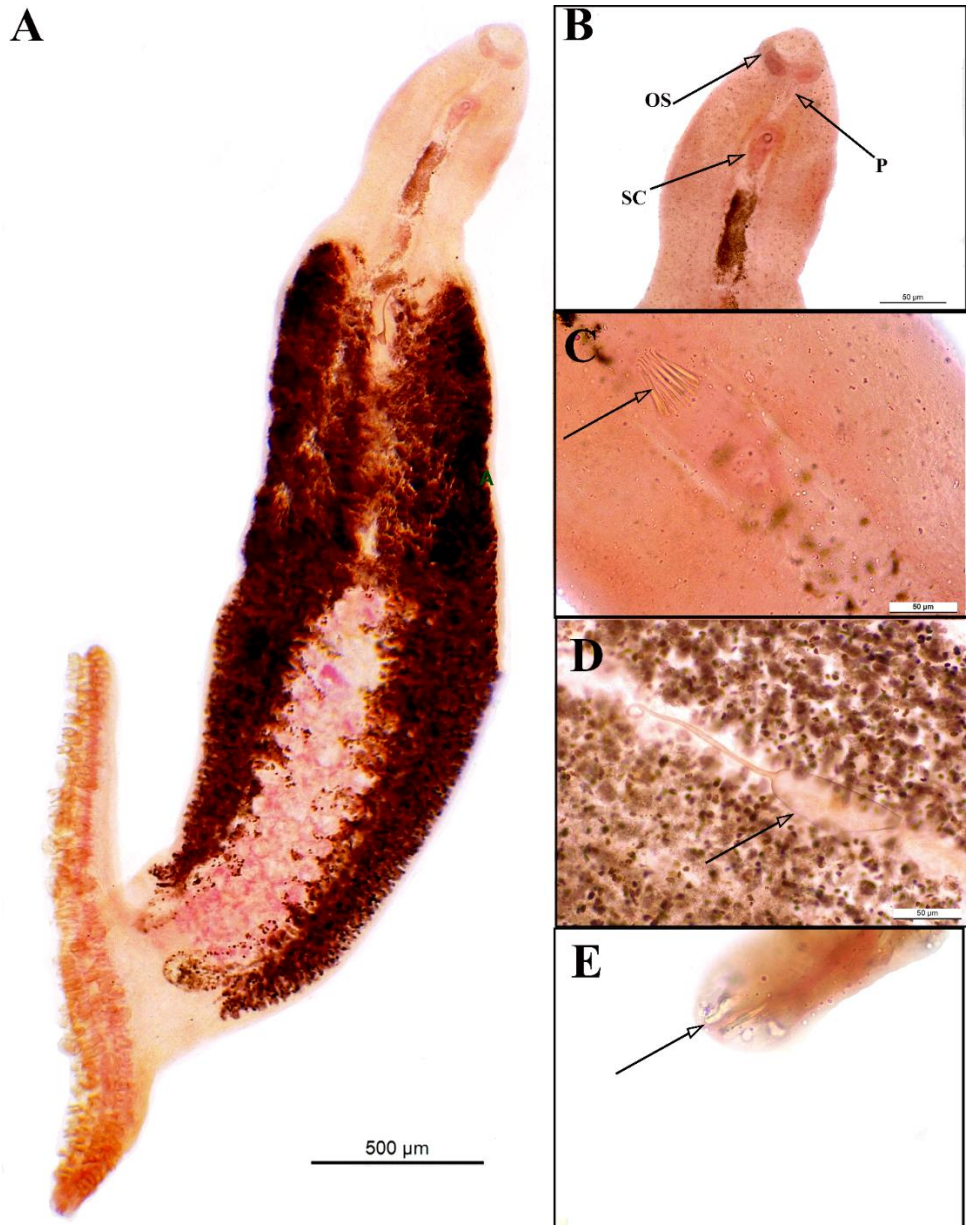

Figure 1 A. whole mount micrograph of Pricea multae B. anterior part of the body os: oral sucker p: pharynx Sc: sclerites of copulatory organ C. Spines of male copulatory organ D. egg E. a pair of anchors.

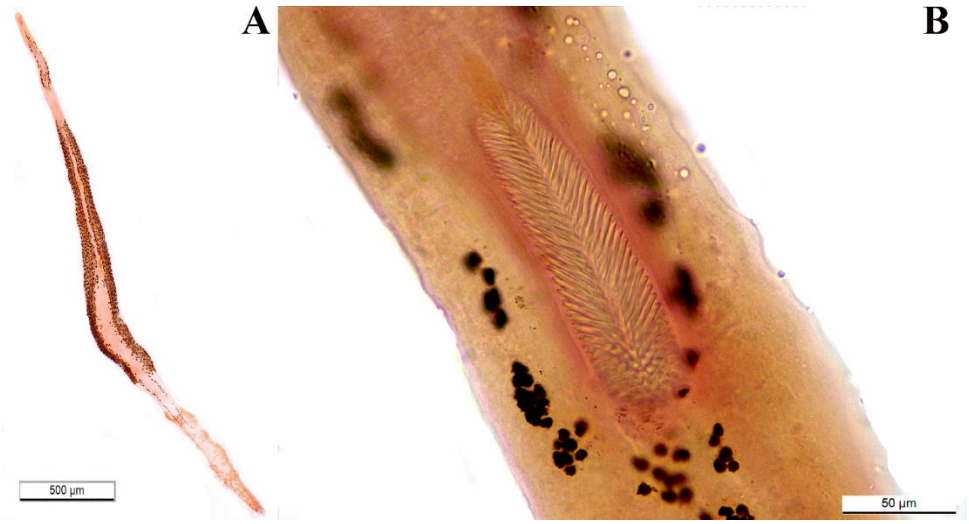

Figure 2 *Gotocotyla acanthura*. A) Light microscopy showing full view. B) Light microscope showing cirrus with pectinate spines around the genital atrium.

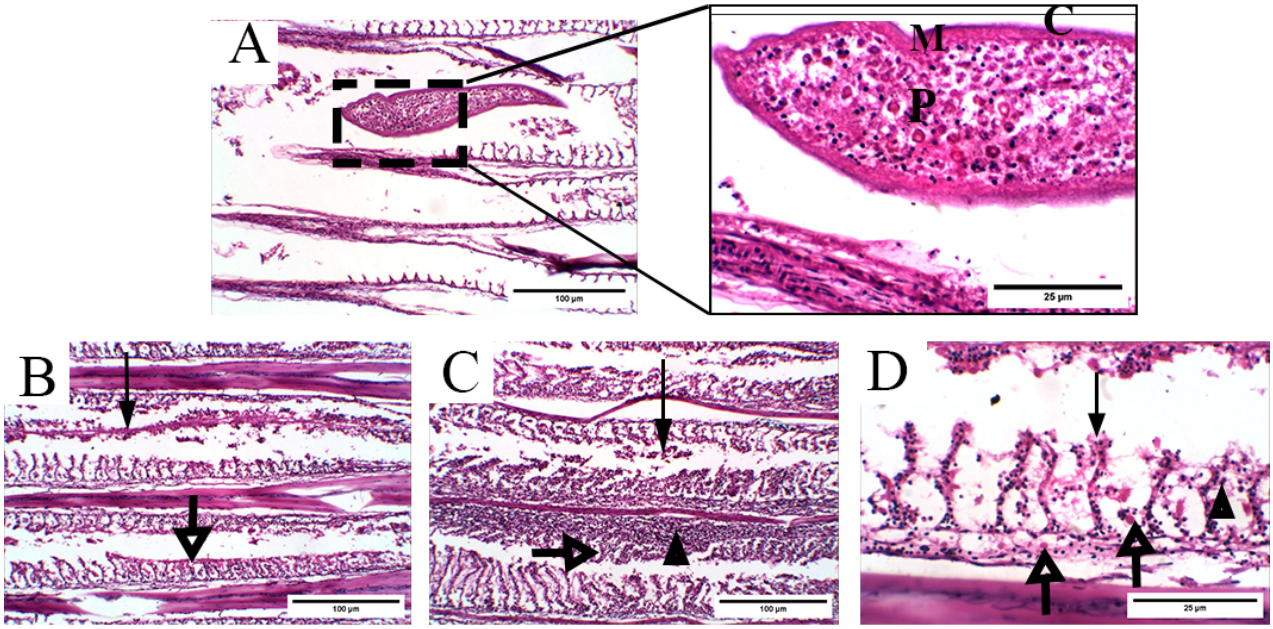

Figure 3. Photomicrograph of H&E-stained infested gill sections showing A) presence of monogeans in interlamellar spaces with massive tip lamellar desquamations, higher magnification on monogeans showing a smooth cuticle (C) with sub-muscular layers (M) and parenchymatous body (P), the adjacent lamellae showed complete loss of secondary lamellae. B&C) Sever lamellar necrosis (thin arrow) with partial to severe lamellar proliferations and fusion (thick arrow) with marked expansion of secondary lamellae by lymphocytic infiltrations (arrowhead). E) Necrotic branchitis characterized by damage of pillar cells with irregularity, desquamation (arrowhead) and lamellar clubbing of secondary lamellae beside lamellar tip hypertrophy (thin arrow) and lymphocytic infiltrations admixed with mucus globules (thick arrows).

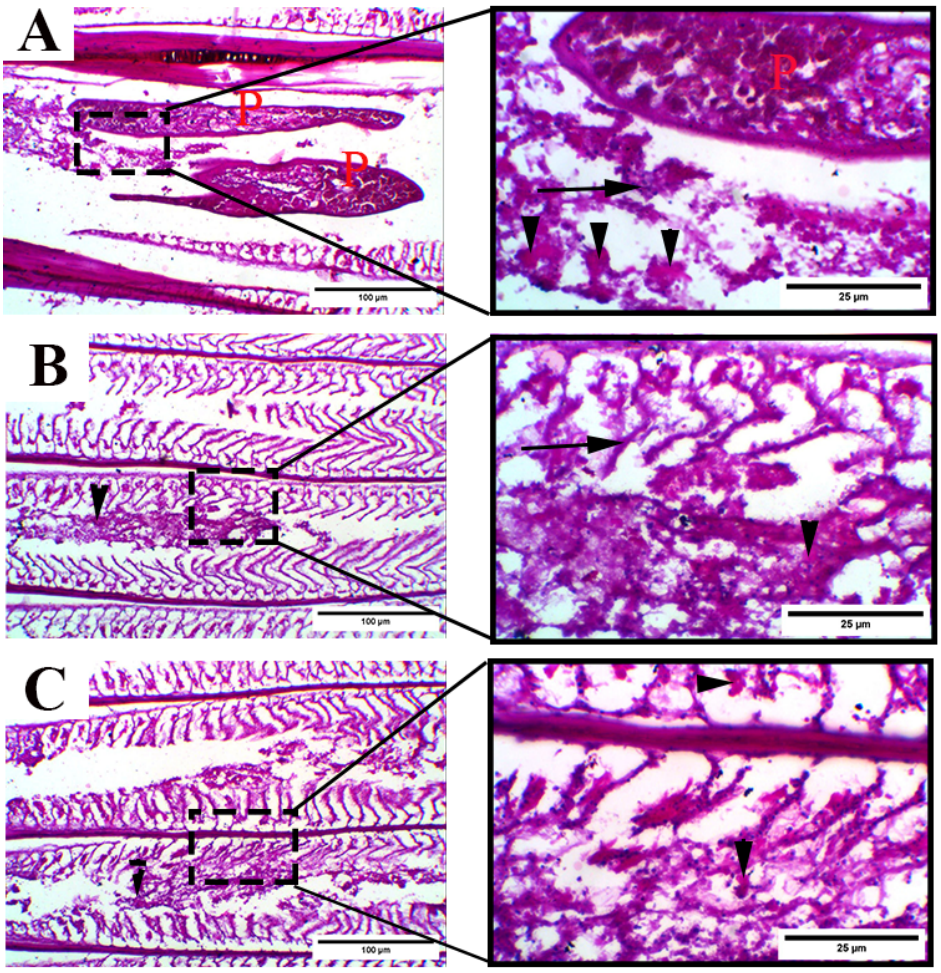

Figure 4. Photomicrograph of periodic acid shiff (PAS) stained infested gills' sections. A) Gills section showing the presence of monogenean parasites (P) in interlamellar space surrounded by excessive dark stained pink patches (arrowheads) accumulated in interlamellar space with necrotic debris, numerous lymphocytes, and macrophages (arrow). B&C) Numerous positive staining mucous cells and mucous globules on base of gills and interlamellar spaces (arrowheads) clumped with cellular infiltrates and sloughed epithelial cells, Higher power showing excessive positive staining mucous cells sloughed from the base of gills with pillar cell damage and secondary lamellar clubbing (arrow) and desquamation beside numerous interlamellar dark red mucoid patches (arrowheads) admixed with focal aggregations of lymphocytes.

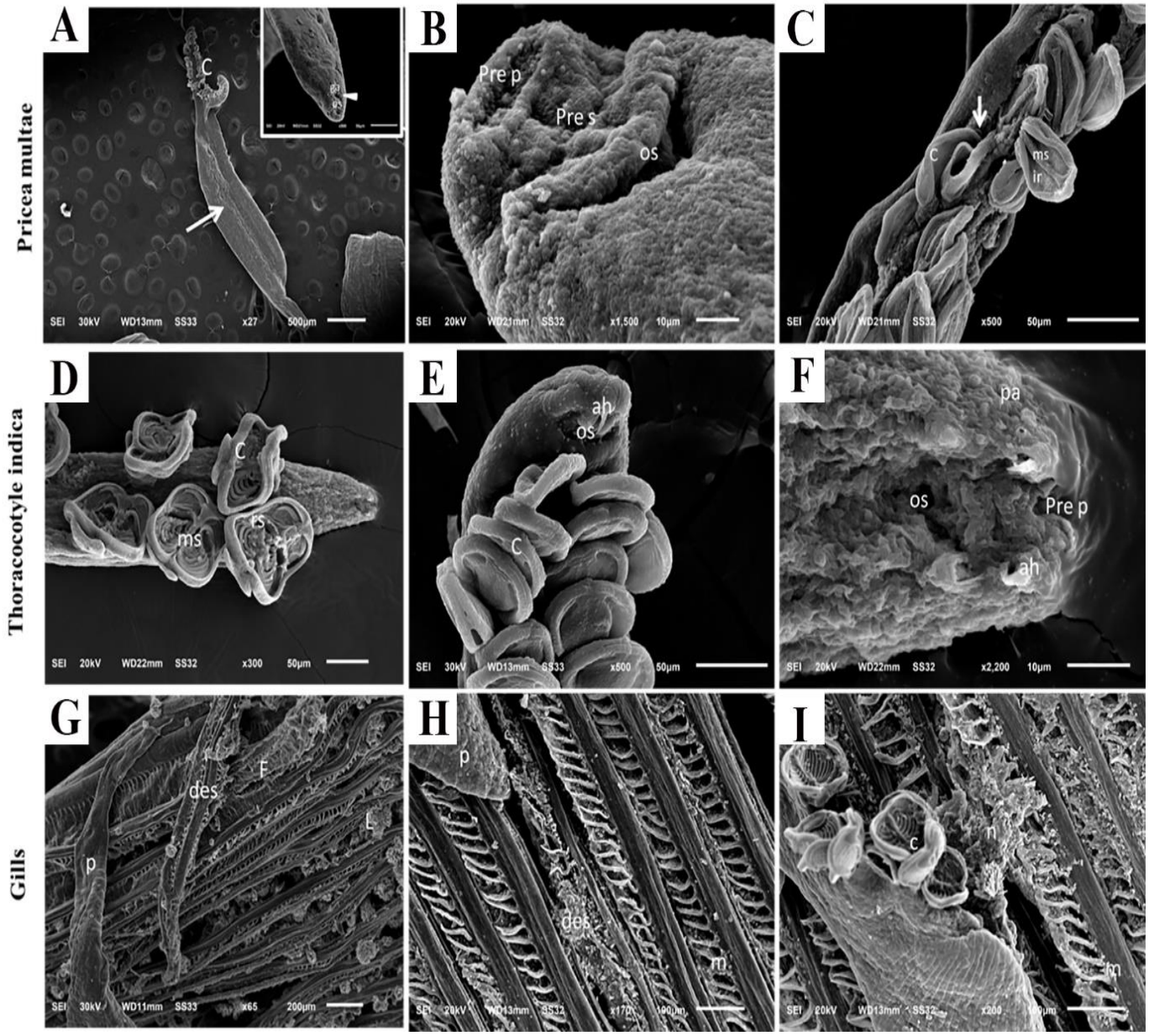

Figure 5. Representative SEM micrograph on monogean parasite structure and their interactions on gills. A) SEM of *Pricea multae* showing flattened transversal ridged tegumental surface (thin arrow) of the whole parasite with posterior clamps (C), inset, anterior portion of the ventral surface showing flattened transversal ridged body with prohaptor, genital atrium (ga) surrounded with microvillous like projection (arrowhead). B) Higher magnification on prohaptor region showing preoral pits (pre p), preoral uniciliated sensilia (pre s) and oral sucker (os). C) The posterior end of *Pricea multae* showing posterior clamps consisted of U-shaped Sclerites with marginal hamulus (thin arrow), inner lateral ribs (ir) and median accessory sclerite (ms). D) SEM of *Thoracocotyle indica* showing a ventral surface of the parasite with ridged nonciliated papillary tegument, numerous clamps of opisthaptor (c) consisting of two rows of clamps with a median sclerite (ms) and a 10 inner rib like sclerite (rs). E) Magnification on the anterior part of the parasite showing proral and oral sucker (os) with anchor hooks (ah). F) More magnification on the anterior part showing a pair of anchor hooks (ah), pro-oral (pre p), and oral opening (os) with ridged dome-shaped tegumental papillae (pa). G-I) Represents the interaction between Thoracocotyle indica and gills, G) Numerous ruffled lymphocytes invading the damaged lamellae and surrounding the attached parasite with lamellar fusion and mucous secretion. H) A flattened, ridged parasite invading an interlamellar opening and resulting in lamellar desquamation (des) admixed with mucous globules (m), cellular infiltrates. I) A transversal ridged parasite attached to lamellar epithelium with opisthaptor clamps (c) and admixed with massive necrotic debris (n) with numerous cellular infiltrates.

4. Discussion

Pelagic fish *S. commerson* feeds mostly on planktonic crustaceans and tiny fish, as do most pelagic fish (Shawket et al., 2018). One type of parasite that lives on fish is called a monogenean. Even though fish are the most frequently affected by parasitic infections, they can also serve as ultimate, paratenic, or intermediate hosts in the parasite life cycle (Ali and Ismail, 2022). In this study, two families of monogenean gill parasites; family Gotocotylidae; *G. secunda*, and family Gastrocotylidae; *P. multae* and *T. indica* were recovered from *S. commerson*. Some different characteristics were observed by using both a light microscope and SEM. Our data showed the characteristic differences in the structure of *P. multae* and *T. indica*. *T. indica* showed the adverse effect of this parasite on the gill tissue using light and SEM. The massive damage to the branchial tissue shows that this parasite is invasive and causes damage to the host tissue. This damage could be attributed to the use of the anterior haptor with its anchor hooks to attach and invade the lamellae. As a response to this attachment, the fish host interacts either by lamellar hyperplasia with the fusion of secondary lamellae or by excessive secretion of mucus (Buchman, 1997). These findings were supported by our H&E and PAS staining results which were confirmed also with SEM. Many previous studies confirmed that the host responds to monogenean attachment by secretion of mucus which is rich in the lysosome, antimicrobial peptides, lectin, and antibodies (Nakamura et al., 2000; Smith et al., 2000). These molecules are attached to parasites and help in immune recognition and attraction of immune cells as lymphocytes and macrophages (Buchmann and Lindenstrøm, 2002).

Concerning the histopathological observations, the monogenean parasites were dispersed in interlamellar spaces mixed with necrotic lamellar epithelial debris with massive destruction of lamellar

epithelium. Additionally, extensive lamellar desquamations with partial to complete lamellar fusion were also seen. The gills were invaded with severe cellular infiltrations composed of numerous lymphocytic infiltrations. These results agree with Arafa et al. (2009) who stated some lesions at the site of attachment a breakdown of epithelial tissue and necrosis besides the fusion of gill lamellae. Additionally, Ali and Ismail (2022) recorded erosions and necrosis of the secondary lamellae with massive mononuclear cell infiltration, and Adawy et al. (2016) found hyperplasia of the epithelium of gill filaments, inflammatory reactions, hemorrhage, destruction of gill tissues due to attachment of monogenean parasites with necrosis of gill filaments and the parasites were easily detected in affected gills. Excessive mucous cells were also observed and disagreed with Igeh and Avenant-Oldewage (2020) who recorded an increase in mucous secretion with neutrophils.

5. Conclusion

The current work provides novel insights into characteristic differences in the structure of *P. multae* and *T. indica* monogeneans parasites in *S. commerson*. The pathological and ultrastructural results revealed massive branchial damage with partial to complete lamellar fusion and lamellar desquamation with complete loss of secondary lamellae and lymphocytic infiltration, the host response includes the appearance of numerous ruffled lymphocytes and mucous globules at the site of parasitic attachment. This damage could be attributed to the use of the anterior haptor with its anchor hooks to attach and invade the lamellae indicating the invasive nature of this parasite.

Conflict of interest: There are no conflicts of interest stated by the authors.

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