

## The Current Situation on Prevailing Parasitic Diseases in *Epinephelus tauvina* (Greasy Grouper)

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### ABSTRACT

This survey was applied to 50 marine fish samples of the greasy grouper *Epinephelus tauvina* collected randomly from Safaga City in the Red Sea province. Infected fish showed no obvious clinical abnormalities except attachment of isopod to the skin. Internally, some fishes showed praniza in the buccal cavity and gills. Other fishes showed dark grayish encapsulated plerocercoid or larvae of philometra in the abdominal cavity and internal organs. The infection rate and intensity of isopods, Praniza larvae, *Philometra* spp., and *Trypanorhyncha* sp. from *Epinephelus tauvina* were 4% & 1/fish, 28% & 3-7/fish, 32% & >20/fish, and 23% & 3-9/fish, respectively. Abundant mixed parasitic infections implicating *Argathona* isopods, Praniza larvae of Gnathiids, Philometrid nematodes, and *Trypanorhynch* larval cestodes, were frequently seen. A morphological description of the structure and characteristics of each isolate was performed. Isolated parasites were identified using optical and electron microscopy to analyze the specimens' morphology. The histopathological alterations in tissues of naturally infected fishes induced by isolated parasites on different organs were described.

### INTRODUCTION

The hammour fish (grouper) is an important source of essential nutrients for humans, providing high levels of protein, healthy fats, vitamins and minerals (Lobna & Abdullah, 2022). Compared to freshwater fish, marine fish are richer in iron, zinc, and vitamin A (Refat *et al.*, 2023). Marine parasites play a significant role ecologically and economically in both wild and farmed fish. Consequently, there is growing interest in the parasitic isopod family Cymothoidae (Khalaf-Allah & Yousef, 2019; Aneesh *et al.*, 2021), even though many species and genera are frequently misclassified or lost. Cymothoid species are protandric hermaphrodites that parasitize a variety of fish species,

feeding on their blood, flesh, and mucus. These isopods can be found on the fish's body surface, fins, mouth, gill chambers, or even within the flesh, having a deleterious impact on fish populations.

Infected fish in captivity may experience inhibited growth, anemia, and even death in smaller fish, resulting in significant economic losses (**Aneesh *et al.*, 2018; Rashid *et al.*, 2021**). Paraniza larvae, found on the gills, fins, and tongue, can cause damage such as fusion of gill lamellae, mechanical injury to the gill epithelium, and necrosis. Severe infestations can lead to the loss of gill function, affecting the fish's survival (**Hassan, 2018**). Philometrid nematodes (Philometridae) are known worldwide and include many species, in marine, fresh, and brackish-water fishes. They can play a significant role in the economy through infection of body cavities and various organs such as musculature, subcutaneous tissues, mouth tissues, and ocular orbits. Moreover, they can also affect fins, gill covers, swim bladder, gill arteries, and body cavity. The importance of *Philometra* spp. parasitizing marine fishes has increased because these pathogenic parasites may cause serious damage to infected fishes or, when parasitic in gonads, significantly decrease fish reproduction and may even cause death to their fish hosts (**Quiazon *et al.*, 2008; Moravec, 2023**).

Larval tapeworms of the Trypanorhyncha family encyst in the coelomic cavity and musculature of teleost fish serve as intermediate hosts. Meanwhile, the adult cestodes are found in the stomach and intestines of fish and marine invertebrates (**Santoro *et al.*, 2020**). The presence of plerocercoids in fish flesh or body cavities creates issues, as it lowers the market value of the fish by making it unappealing to consumers. Additionally, Trypanorhyncha larvae in the musculature may release toxins that can affect humans who accidentally consume raw, undercooked, or poorly preserved fish, leading to allergic reactions (**Morsy *et al.*, 2022**).

This research aimed to gather comprehensive information on the prevalence of common parasitic diseases affecting *Epinephelus tauvina* from Safaga City. It also sought to examine the clinical symptoms and post-mortem lesions associated with the infection. Additionally, the study aimed to confirm the identification through scanning electron microscopy and to highlight the histopathological changes in fish naturally infected with parasites.

## MATERIALS AND METHODS

### 1. Sampling: Naturally infected fish

A total of 50 healthy marine fish samples of *Epinephelus tauvina* (Greasy grouper) were randomly collected from Safaga City in the Red Sea province between June 2022 and March 2023. The fish ranged in size from 35 to 42cm in length and weighed between 550 and 1250 grams. They were then promptly transported to the laboratory of the Ismailia branch of the Animal Health Research Institute.

## **2. Clinical picture**

Clinical and postmortem examinations were made externally and internally on the life or freshly dead fish for detection of any clinical abnormalities (Noga, 2010).

## **3. Parasitological examination**

The fish were examined both macroscopically and microscopically to detect parasites. Isopod specimens were studied using a dissecting stereomicroscope and light microscopy after being cleaned with a warm saline solution. The specimens were then preserved in 70% ethyl alcohol. Identification was carried out using modern classification keys based on morphological characteristics, following the guidelines of Aneesh (2014). Paraniza larvae were collected from the gills, and mouth of fish, then washed several times with physiological saline to be free from mucus. The larvae were preserved in 70% ethyl alcohol and were then dissected and mounted in lactophenol as temporary slide preparations fixed using the techniques described by Lucky (1977).

The recovered nematodes were rinsed in physiological saline for light microscopic examination (LM) and were then cleared in glycerin. The larvae collected from each site were washed in physiological saline, relaxed, and fixed in a hot alcohol-glycerin solution (5%) until the alcohol evaporated, leaving the specimen in almost pure glycerin. The specimens were then cleared in lactophenol, mounted in glycerin gelatin according to the method of Meyer and Olsen (1992), left to dry, and examined microscopically. Nematode identification was carried out following the method of Ramachandran (1973).

The capsulated plerocerci were extracted from the infected organs, and the walls of the parasite blastocysts were opened to remove the juvenile scolices. The specimens were rinsed in saline solution and fixed in 10% buffered formalin. They were then stained with acetic carmine, dehydrated, and mounted in Canada balsam. The collected cestodes were identified using the identification key provided by Yamaguti (1959, 1961).

## **4. Scanning electron microscopy**

The specimens were cleaned after being rinsed in tap water. They were fixed in equal volumes of 4% glutaraldehyde and 0.2% cacodylate for two hours and processed as described by Eissa *et al.* (2022), and then examined using a scanning electron microscopy (JSM-IT100 InTouchScope™ Scanning Electron Microscope, JOEL, Damansara, Selangor, Malaysia).

## 5. Histopathological analysis

For the histopathological study, the fish were euthanized, and tissue specimens of liver, spleen, and skin were excised, rinsed in normal saline, and fixed in formalin buffer 10% for 24h. After fixation, the tissues were dehydrated in alcohol series of ascending concentration (70, 80, 90, and 100%, respectively), cleared with xylol, embedded in paraffin, blocked, cut/sectioned at 5µm sections, The tissue sections were stained with hematoxylin-eosin (H&E) according to the method described by **Bernet *et al.* (1999)**.

## RESULTS

### 1. Clinical findings

The naturally infected marine fish exhibited no visible clinical abnormalities externally, except for the presence of isopod infections, which could be seen with the naked eye attached to the skin, causing abrasions and small wounds (Fig. 1A, B). Internally, Paraniza larvae appeared as small black or grey dots attached to the gills or in the buccal cavity, accompanied by excessive mucus secretion, which sometimes caused lamellae adhesion (Fig. 1C, D). Endoparasites were found in the body cavity and internal organs of *Epinephelus tauvina*, including reddish to black, comma-shaped nematodes (Fig. 2B, C, D) and some grayish to black encapsulated plerocercoid larvae in the branchial cavity and internal organs (Fig. 2A, B, D). Infected fish with nematodes displayed orange, brownish, or black spots on the mesentery, mesenteric fat, and internal organs such as the liver, intestine, and gonads, either sporadically or in clusters forming patches (Fig. 2E).

### 2. Parasitological findings

#### 2.1. Morphological description of the collected isopod

*Argathona* spp. (Family Corallanidae, *Argathona Stebbing*, 1905) were found attached to the skin resulting in abrasions with small wounds, with a prevalence rate of 4%. Corallanidae with antennule peduncle biarticulate, basal article not expanded. With prominent frontal lamina, lateral margins clypeus backwardly produced, labrum is encompassing, narrow cutting edge mandible, large palp, and molar process was observed. Maxillule with exopod was terminated in an unguis-like point, endopod with truncate lobe. Maxilla consisted of a single simple lobe. Maxilliped has 5-articulate palp, showing all degrees of fusion between articles 2 and 3. Pereopods 1-3 with dactyls sub-prehensile without digitiform processes, pereopods 4-7 ambulatory. All Pleopods were similar (Figs. 3A, B, C & 4).

#### 2.2. Morphological description of *Praniza* larva, Family Gnathiidae

The larvae were found in the buccal cavity and gills, occurring in 14 out of 50 cases (28%). The body is elongated and divided into three sections: the cephalosome,

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pereon, and pleon. The cephalosome contains the antennae and mouthparts, the pereon has six pairs of pereopods, and the pleon features pleopods (Fig. 3D)

**2.3. Morphological description of the collected nematode**

*Philometra* spp. (Family: Philometridea, Baylis & Daubney, 1926) were scattered, and heavy infections were distributed and embedded in mesenteric tissue of the abdomen and internal organs such as the liver and stomach with a prevalence of 32%.

*Philometra* spp. are characterized by a filiform body, round and smooth cephalic end smoothed cuticle, long esophagus, and tapering posterior end (Figs. 5A, B, C, D & 6)

**2.4. Morphological description of the collected cestode**

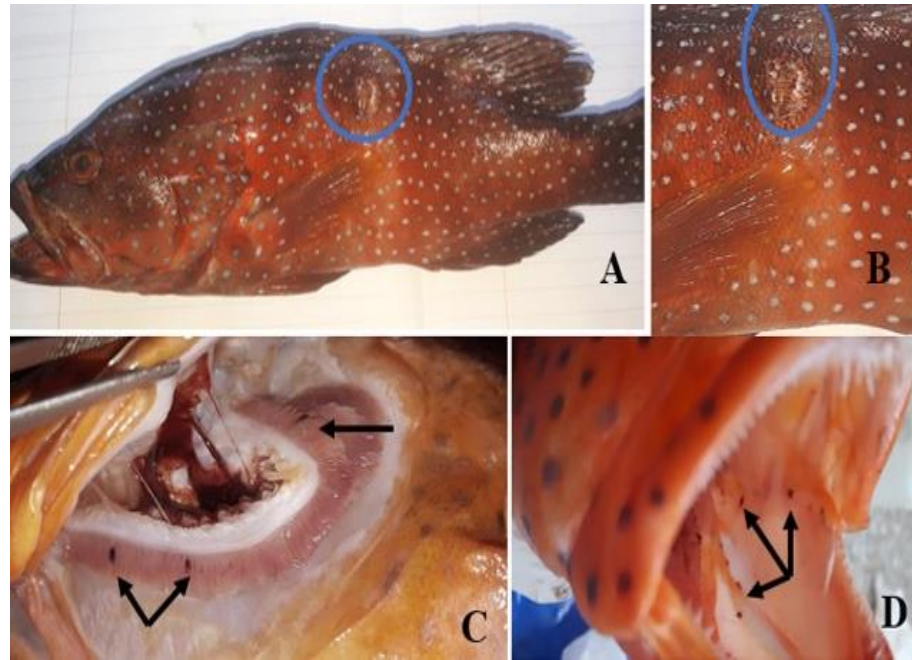
**Encapsulated plerocercoid *Trypanorhyncha* spp.**

Blastocysts were observed in the branchial and the abdominal cavity, surrounded and adhered to the peritoneum, mesenteric fat, and abdominal organs with a prevalence rate of 23%. Internally numerous multifocal to coalescing parasitic cysts were attached or deeply embedded within the peritoneum and adhered to the serosal surfaces of the abdominal organs and gills, often extending into the muscularis. These cysts were irregular, circular to oval with a narrowed end, variably sized grayish to black in color, larvae-filled, with small amounts of serous fluid. Concurrently, numerous multifocal to coalescing dark nodules were embedded within the fibrous tissue and adhered to the serosal surfaces of the celomic organs. The plerocercus was enclosed within an oval-shaped blastocyst (Figs. 6: E, F & G, 7). The scolex was found inside the blastocyst with four long cylindrical tentacles with coiled tentacle sheaths. Four symmetrically arranged bulbs were observed at the end of a scolex.

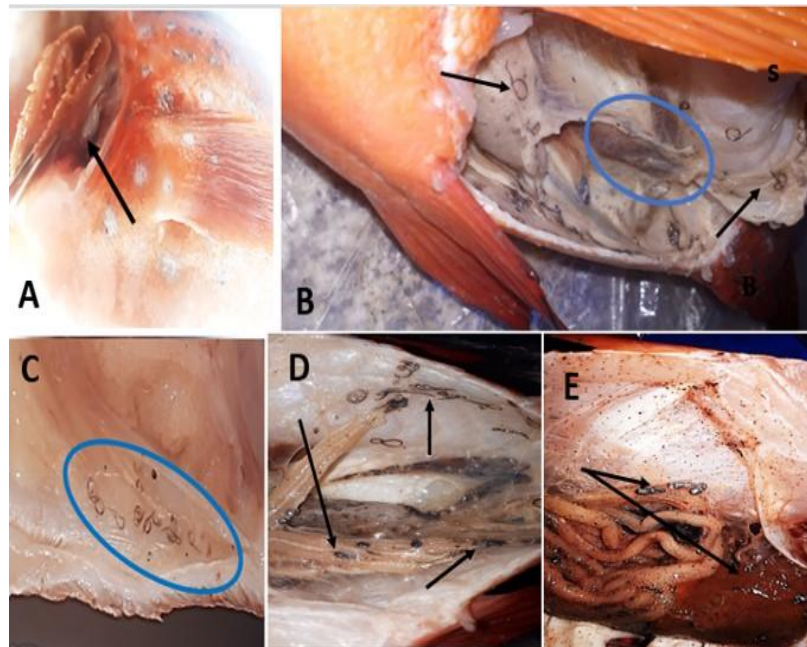
**Table 1.** The prevalence of parasitic infection in examined *Epinephelus tauvina*

Parasites	Prevalence	Site of Infection	Intensity of infection	Mixed infection			
				N	C	I	P
Isopods (I)	4 %	Skin	1	+/-	/+-	_	+/-
Praniza larvae (P)	28 %	Buccal cavity, gills	3-7	+/-	+/-	+	_
Nematodes( <i>Philometra</i> ) N	32 %	Mesentry, liver, stomach, abdominal cavity	Heavy infection	+	+	+	+
Cestodes( <i>Trypanorhyncha</i> ) (C)	23 %	Branchial cavity, abdominal cavity	3-9	+/-	_	+	+

+ present with, - not present alone, +/- with or without

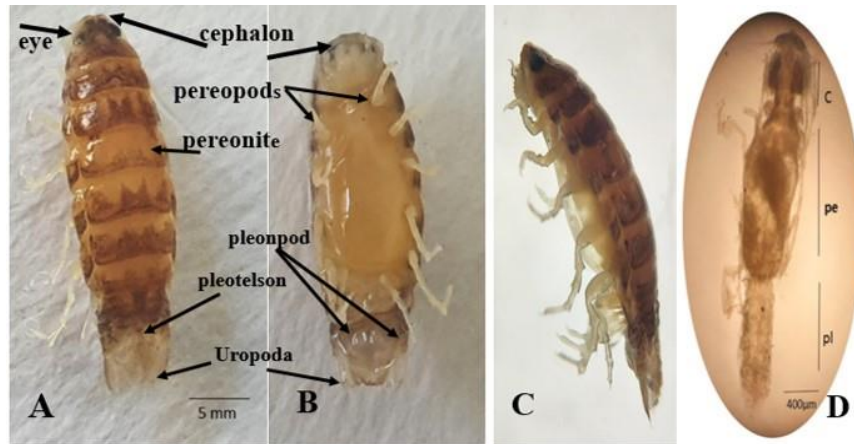


**Fig. 1.** *A.* *Epinephelus tauvina* showing *Argathona* isopod attached to the skin. *B:* Higher magnification of the isopod attached to the body surface. *C:* Infested gills showing numbers of *Praniza* larvae appear as black dots with excessive mucous. *D:* *Praniza* larva in buccal cavity (arrow)

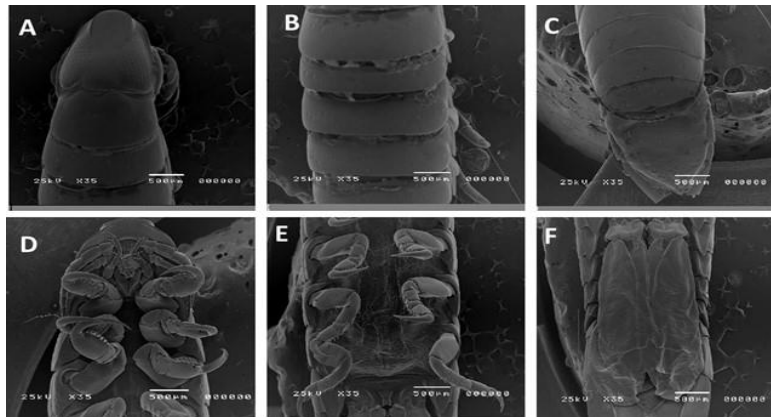


**Fig. 2.** *A.* *Epinephelus tauvina* infected with plerocercoids in the branchial cavity (arrow) *B:* infected fish showing dark grayish Encapsulated plerocercoid and blackish philometra in the abdominal cavity. *C:* Numerous nematodes in abdominal muscles. *D:* Nematodes & plerocercoid in abdominal cavity. *E:* Greasy grouper fish showed orangish, brownish-to-black spots on the mesentery and internal organs

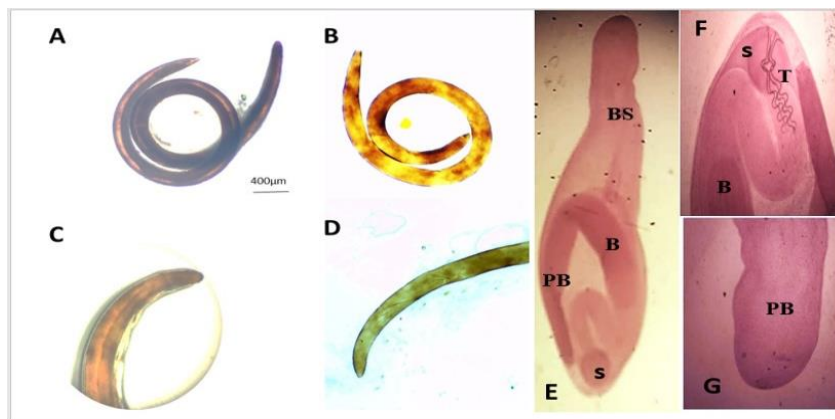
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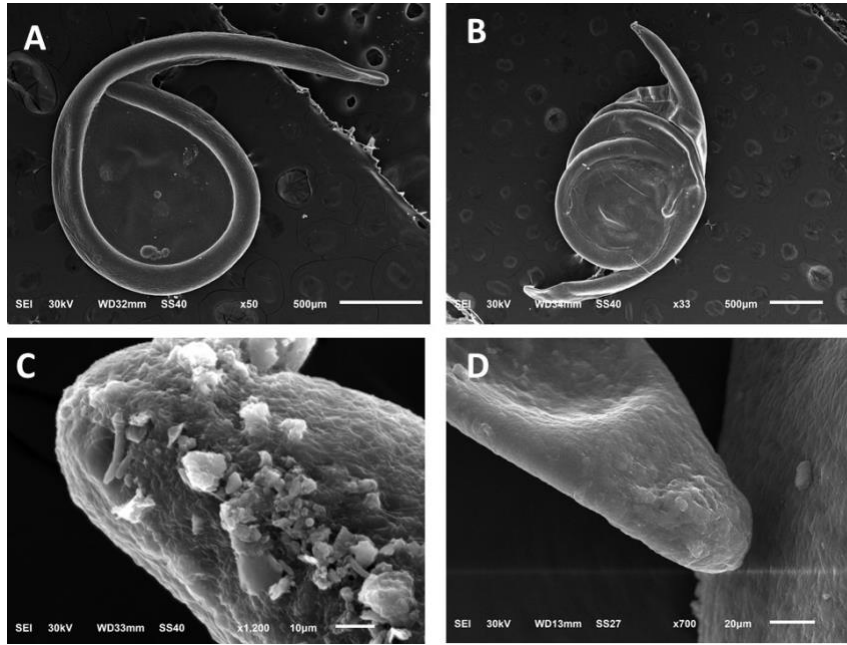
**Fig. 3.** A, B. Dorsal and ventral surface of *Argathona* spp. C: dorsal and ventral view. D: LM for praniza larva of *Gnathia* species



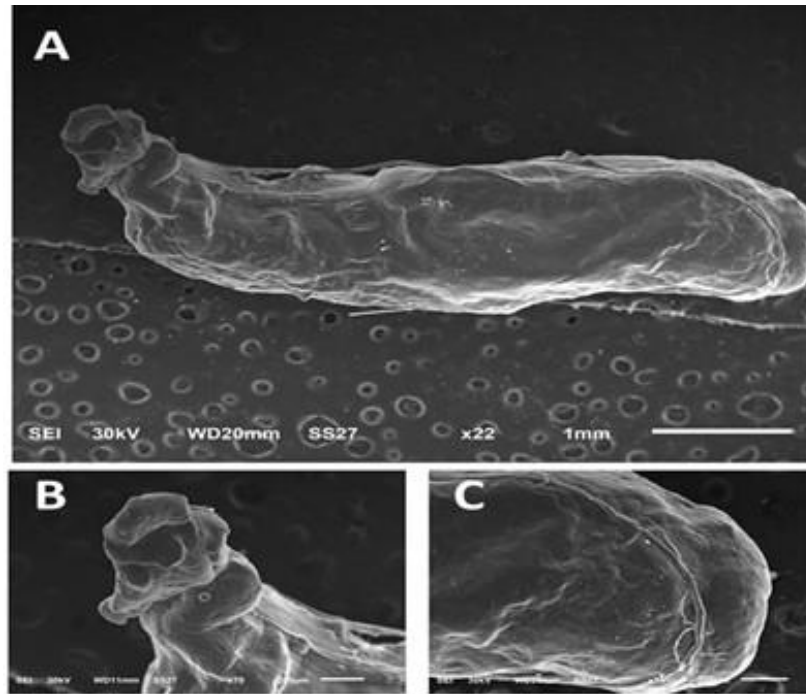
**Fig. 4.** Scanning electron microscopy of *Argathona* spp. A, B, C: dorsal surface. D, E, F: ventral surface



**Fig. 5.** A, B. LM of whole *Philometra* spp., C: anterior end, D: posterior end of the body. E: LM of *Plerocercus* within the blastocyst. F: Scolex of the plerocercus. G: Postbulbosa of the plerocercus. BS: blastocysts, S: Scolex, T: tentacles, B: bulbs, PB: postbulbosa



**Fig. 6.** **A, B:** Scanning electron micrographs of *Philometra* spp. from grouper fishes, *Epinephelus tauvina*. **C:** Anterior end. **D:** Posterior end of body



**Fig. 7.** **A, B.** Scanning electron micrographs of a plerocercoid within the blastocyst **B:** Anterior part. **C:** Posterior part



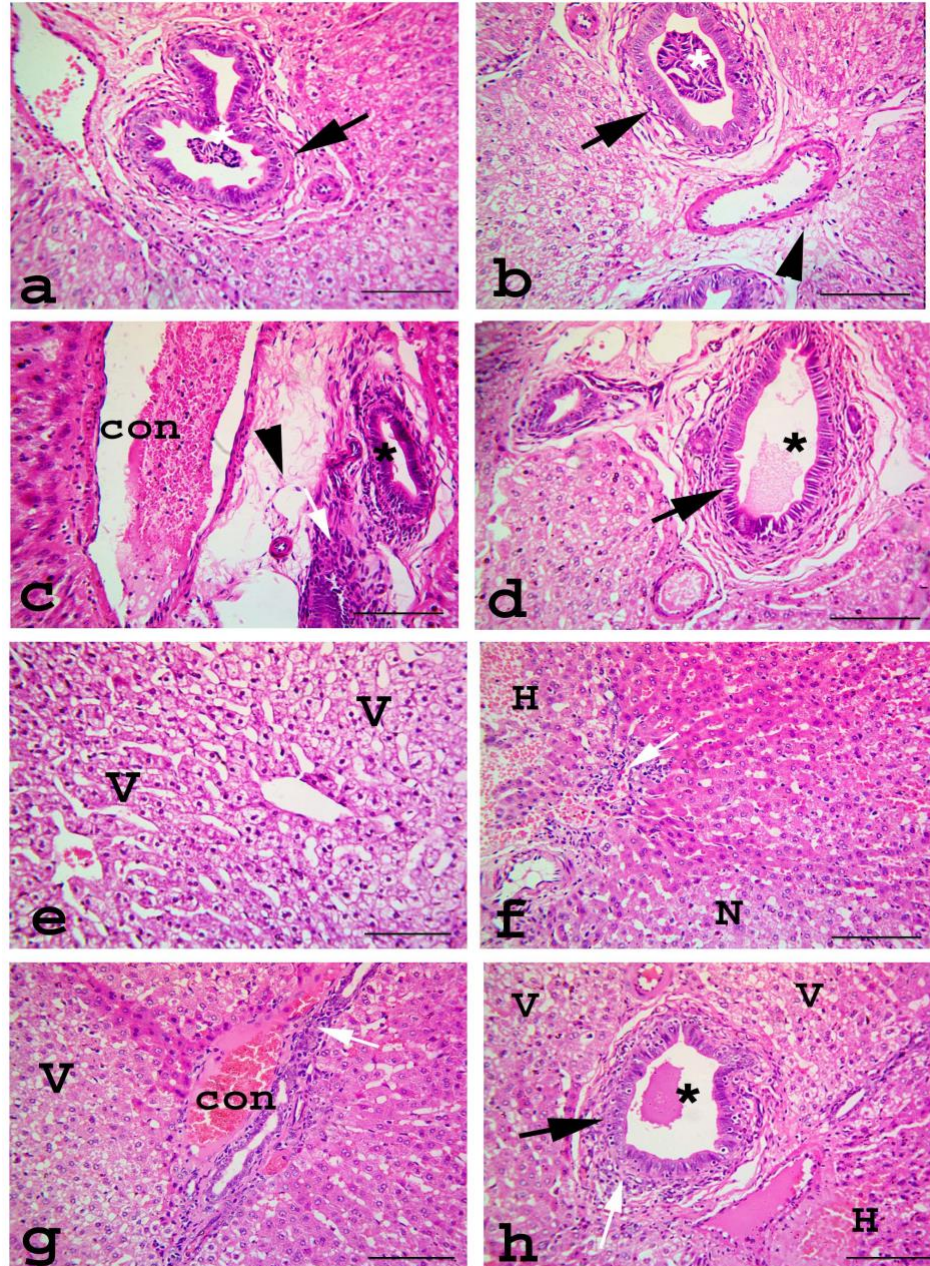
### **3. Histopathological results**

Histopathological examination of the infected liver showed multiple parasitic cysts embedded in hepatic parenchyma. The cyst may be viable or destructed. The viable cyst was formed from scolex, internal structure, cuticle, and cyst membrane with comparatively thick fibrous capsule. Some degenerated cysts were detected, revealing distorted parasitic elements, and parts of the cyst membrane, with reactive fibrous capsule, sanguineous exudate, and inflammatory cells.

Portal aggregation of inflammatory cells and Periportal fibrosis were also observed. Severe congestion of hepatic vessels, and severe fatty and hydropic degeneration were detected with multifocal areas of necrosis, leukocytic infiltration, and a local extensive area of hemorrhage (Fig. 8).

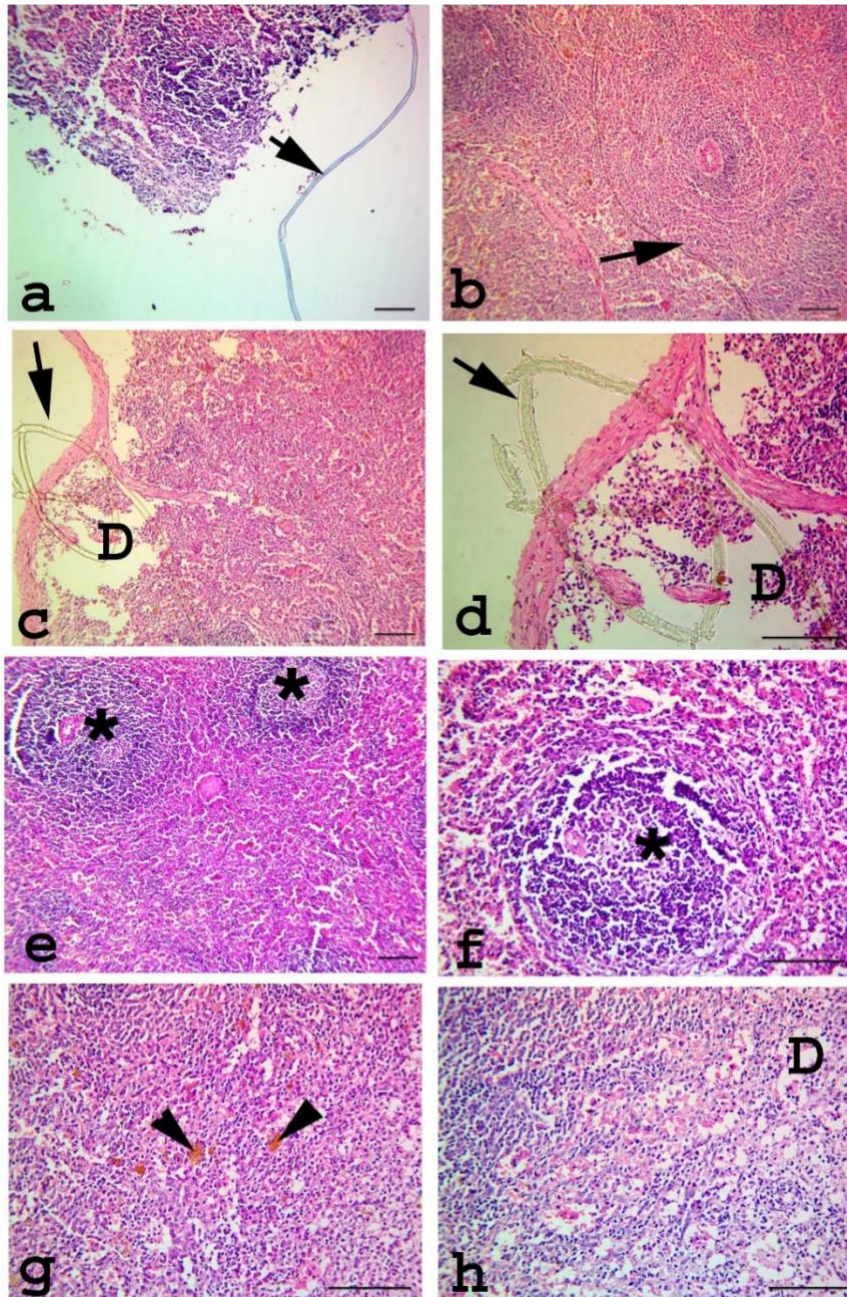
Although, the infected spleen of *Epinephelus tauvina* revealed long thin nematode worms embedded in splenic tissue. Splenic tissue showed a multifocal area of severe lymphoid depletion, with a well-distinct melanomacrohage center (MMC) in response to parasitic infection. The spleen also showed multiple granulomas. Granuloma showed aggregation of many macrophages and lymphocytes, the whole granuloma encapsulated with a delicate layer of fibrous connective tissue, replacing and displacing a large area of the splenic parenchyma. (Fig. 9).

Histopathological observations of the skin of infected fish indicated that the parasites were present in the superficial layers as well as in the deeper layers of the epidermis. Skin tissue showed aberrant conditions including erosion, destruction, separation, dislocation, and shrinkage. In the dermis, collagen fibers were separated from each other with severe leucocytic infiltration and multifocal area of hemorrhage. At higher magnification, the morphology and architecture of the skin appear altered with erosion. The infected skin showed severe damage to both epidermis and dermis. (Fig. 10).

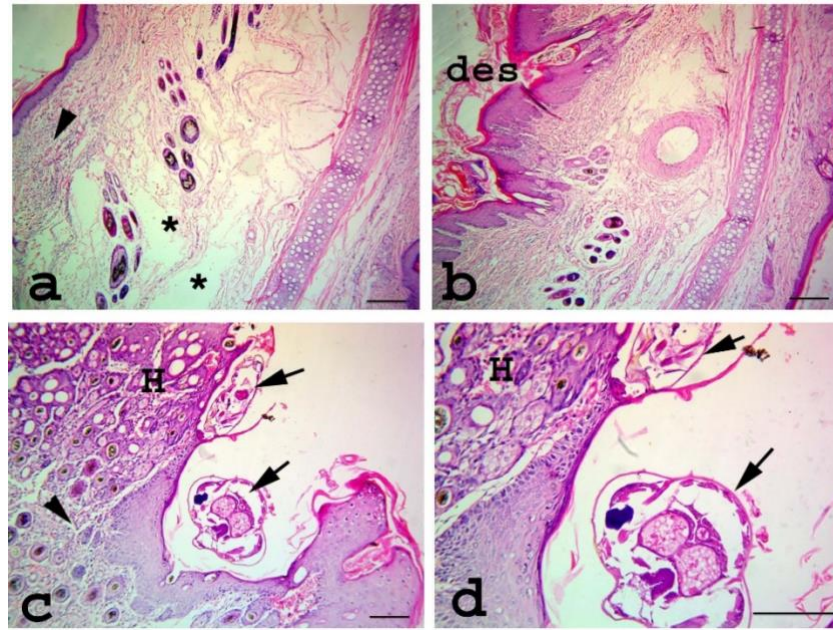


**Fig. 8.** Photomicrograph of hepatic tissue of *Epinephelus tauvina*, infected with plerocercoids (a-b) showed viable parasitic cyst (white asterisks) with thick fibrous capsule (black arrow) and vacuolation of hepatocytes (V). (c-d) showed degenerated parasitic cysts (black asterisks), severe congestion of portal vessels (cong), sanguineous exudate (arrowhead), and severe inflammatory cells (white arrow). (e) showed severe vacuolation of hepatic cells (V). (f) showed areas of necrosis (N), severe hemorrhage (H), and vacuolation of hepatic cells (V). (g) showed congestion of portal vessels (cong), vacuolation of hepatic cells (V), and leukocytic infiltration in the portal area (white arrow). (h) showed degenerated parasitic cysts (black asterisks), hemorrhage (H), and vacuolation of hepatic cells (V). Hematoxylin and eosin. X40. bar = 100 $\mu$ m

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**Fig. 9.** Photomicrograph of splenic tissue of *Epinephelus tauvina*, infected with *philometra* (a-d) showed a long nematode embedded in splenic parenchyma (black arrow) with severe lymphoid depletion (D). (e-f) showed multiple granulomas embedded in splenic parenchyma (black asterisks). (g) showed prominent melanomacrophage centers (arrowhead). (h) showed lymphoid depletion (D). Hematoxylin and eosin. bar = 100µm



**Fig. 10.** Photomicrograph of skin of *Epinephelus tauvina*. infected with isopods. **a:** showed widely separated collagen fibers of the dermis from each other (black asterisk), with severe leucocytic infiltration (arrowhead). **b:** showed severe destruction of superficial and deep layers of the epidermis (des). **c-d:** Protozoans were found at the surface as well as penetrated in the deeper layers of the epidermis (black arrow), with an area of hemorrhage(H) and leucocytic infiltration (arrowhead). Hematoxylin and eosin. bar - 100 $\mu$ m

## DISCUSSION

A variety of parasite species affect marine fish, they are present in several kinds of tissues and organs, resulting in severe economic losses (Tadros *et al.*, 2020). In the present study, a total of four different parasite species (*Argathona* sp., Praniza larvae, *Philometra* sp., and *Trypanorhyncha* sp.) were found, no gross lesions were revealed externally, except for isopod infestations, which can be visibly spotted on the skin, leading to abrasions and tiny wounds. This result was similar to those reported by Purivirojkul and Songsuk (2020) and Abdulmohsen *et al.* (2024), who found small white isopods attached externally to the skin, and mouth cavities. The postmortem findings are similar to that recorded by Lamie and Abdel-Mawla (2015), who found praniza larvae as small black or grey dots attached to gills or on the buccal cavity with excessive mucus secretion which leads to adhesion of lamellae in some cases. Additionally, the current results match those of Purivirojkul and Songsuk (2020), who isolated the praniza stage from 15 species of fishes in the Gulf of Thailand (*Epinephelus areolatus*, *E. coioides*, *E. erythrurus* and *E. quoyanus*).

The endoparasitic nematodes, *Philometra* were detected in *Epinephelus tauvina* as a reddish to black color coma-shape. These results are congruent with those of Abo-Esa

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**and Abdel-Mawla (2012)**, who recovered the reddish *Philometra* sp. from the ovaries of *Lethrinus lentijan*, and **Moravec and Bakenhaster (2021)**, who isolated 2 types of *Philometra* from *Tylosurus crocodilus*. The orange, brownish, and black spots on mesentery, mesenteric fat and internal organs such as the liver, intestine, and gonads, sporadic or in clusters making patches observed in this study in case of infection by *Philometra* or mixed with plerocercoid as a result of an allergic reaction of fish. These findings are agreeable with the findings of **Adel (2013)**, who observed granulomatous reaction by the host tissue against the parasite, mononuclear inflammatory cells, and eosinophilic granular cell infiltration, together with necrosis of the pancreatic tissue, which affect fish marketability. A similar study was performed by **Attia et al. (2021)**, who recorded granulomatous reaction in the skeletal muscles of orange-spotted grouper (*Epinephelus coioides*) infected with *Huffmanella* spp. The inflammatory response appeared as a chronic inflammatory reaction in the form of granuloma formation between the muscular tissue in some fish species infected with the nematodes parasite .

Meanwhile, encapsulated plerocercoids were detected as a grayish-to-black color. This result concurs with that obtained by **Abo-Esa and Badawy (2006)**, **Al-Zubaidy and Mhaisen (2011)**, **Abo-Esa and Abdel-Mawla (2013)**, **Abdel-Mawla et al. (2015)**, **Abd El Maged et al. (2021)** and **Morsy et al. (2022)**. Trypanorhycha larvae infect a diverse range of marine invertebrates mostly using fish as intermediate or paratenic hosts. The infected fish may represent marketing problems for commercially exploited species causing economic losses only a few cases of accidental human infections by trypanorhynch have been reported when eating raw or poorly cooked fish containing plerocercoid, causing allergic reactions (**Abdelsalam et al., 2016; Kuraieem et al., 2016**).

In the examined fish, the prevalence rate of isopods was 4%, which is similar to the results obtained by **Eissa et al. (2020)**, who reported a prevalence of 4.54% in marine fish infected in the Suez Canal, Ismailia province. However, this rate is lower than that recorded by **El-Lamie and Abdel-Mawla (2015)**, who found a prevalence of 10.7% in the skin of *Mugil capitus*, and by **Zayed et al. (2023)**, who reported 16.1% in various fish species from the Egyptian waters of Alexandria. Similarly, **Abdulmohsen et al. (2024)** isolated isopods with a prevalence of 16% from *Epinephelus chlorostigma* in Suez Governorate. Conversely, our result is higher than the prevalence recorded by **Purivirojkul and Songsuk (2020)**, who found *Argathona macronema* on the skin of *Epinephelus coioides* with a prevalence of 1.48%. However, it remains lower than the prevalence of *Argathona rhinoceros*, which was recorded at 7.41% in the nasal and branchial cavities of the same fish. Variations in fish species, parasite types, and the sampling locations may explain these discrepancies.

Meanwhile, the prevalence of *Gnathia* sp. (praniza larvae) was 28%. This result closely aligned with the findings of **El-Shaer and Sallam (2023)**, who reported a

prevalence of 32% in *Mullus surmulatus*, and those of **Zayed *et al.* (2023)**, who recorded 27.1% in *Scomberomorus commerson*. However, it differed from the findings of **El-Lamie and Abdel-Mawla (2015)**, who detected a prevalence of 10% in *Siganus revulatus* and 50% in *Scarus* sp.

In cases of heavy infestation, praniza larvae may cause severe economic losses in cultured fish due to their impact on vital respiratory organs and their high saturation with blood. This can lead to hypoxia as a result of gill function loss. Additionally, *Hemogregarine bigeminal*, a protozoan blood parasite in fish, can be transmitted by praniza larvae (**Hassan, 2018**).

The prevalence of *Philometra* spp. in *Epinephelus tauvina* was 32%, which closely matches the finding of **Abo-Esa and Abdel-Mawla (2012)**, who reported a prevalence of 34.2% in *Lethrinus lentijan* from the Red Sea. However, this result differed from the findings of **Moravec and Bakenhaster (2021)**, who isolated two species—*Philometra barracudia notabilis* and *Philometra consimilis*—with prevalence rates of 20 and 80%, respectively, from *Tylosurus crocodilus* on the Atlantic coast of Florida.

The prevalence observed in this study is also lower than the 50% prevalence recorded in the ovaries of *Epinephelus* spp. but higher than the 20% recorded in the ovaries of *Saurus tumbil* from the Red Sea (**Tantawy & Abo-Esa, 2007**). Furthermore, it exceeds the rates reported by **Mohamed *et al.* (2010)** from *Epinephelus tauvina*, *Lethrinus nebulosus*, and *Epinephelus chlorostigma* in the Arabian Gulf (24.13, 21.87, and 20%, respectively) and **Moravec *et al.* (2021)**, who recorded 5% in the ovaries of *Lutjanus lutjanus* and 3% in the ovaries of *Platycephalus indicus*. These variations may be attributed to differences in climatic conditions, host species, fish size, and sampling periods.

Regarding the prevalence of *Trypanorhyncha* sp. (23%), the results are similar to those obtained by **Al-Zubaidy and Mhaisen (2011)**, who reported a prevalence of 24.3% in the body cavity of certain Red Sea fish, as well as **Abo-Esa and Abdel-Mawla (2013)**, who recorded 20% in *Trachurus indicus*, and **Abdel-Mawla *et al.* (2017)**, who observed 24% in *Sphyræna jello* and 28% in *Trachurus indicus*. However, the prevalence in this study is lower than that reported by **Abo-Esa and Badawy (2006)** for *Hamour* fish (66.6%) and **Abo-Esa and Abdel-Mawla (2013)** for *Saurida undosquamis* (70%) and *Sardinella* sp. (40%). It is also lower than the findings of **Mahmoud *et al.* (2015)**, who recorded prevalence rates of 46%, 44%, and 36% in *Epinephelus gigas*, *Sciaena umbra*, and *Scomber* spp., respectively. Additionally, it is lower than the rates reported by **Abdel-Mawla *et al.* (2017)** for *Scomberomorus commerson* (30%), **Morsy *et al.* (2022)** for *Epinephelus haifensis* (76.5%) and **Abd El Maged *et al.* (2021)**, who recorded 44% in the grouper fish. These differences may be ascribed to variations in the localities where the fish specimens were collected, the species examined, and the types of parasites detected.

## **CONCLUSION**

The current study highlights the clinical picture, morphological features, and the associated pathological impact of the parasites infecting the greasy grouper from Safaga City in the Red Sea province. Although, the previous results indicated that the infected *Epinephelus tauvina* had no pathognomonic clinical abnormalities, except isopoda. The highest infection was *Philometra* spp., followed by pranzia larvae, *Trypanorhyncha* and isopod *Argathona*. Moreover, the infected fish are marketing repulsive with economic losses. Confirmed identification was done using optical and electron microscopy to analyze the specimens' morphology. The histopathological alterations in tissues were described in the skin, liver, and spleen.

## **ETHICAL APPROVAL**

This study was approved by the Ethics Committee of Suez Canal University. All animal experiments were conducted following the guidelines of the Guide for the Care and Use of Laboratory Animals, Faculty of Veterinary Medicine Science, Suez Canal University, Egypt (Approval No. 2022050).

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