

**Identification and Histopathological Study of Trematode, *Gonapodasmius epinepheli* in Orange Spotted Grouper, *Epinephelus coioides* (First report from Persian Gulf, Iran)**

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**Abstract**

*Gonapodasmius epinepheli*, a didymozoid trematode, was found in orange spotted grouper (*Epinephelus coioides*, Hamilton-Buchanan, 1822), in Khark Island coastal area in Persian Gulf, Iran. The *Gonapodasmius epinepheli* encysted on gills in primary lamellae. Histopathological serial sections revealed that larvae were mainly embedded under the gill epithelium and eggs of the parasite were disseminated in some organs such as gill epithelium and uterus lumen. Pathological changes and host response were primary reduction and destruction of secondary lamellae. Various stages of the life cycle of this parasite were discussed in this study.

**Keywords:** *Gonapodasmius epinepheli*, *Epinephelus coioides*, Trematode, Orange spotted grouper

**Introduction**

Orange-spotted grouper, *Epinephelus coioides* has recently become one of the most important fish for culture and trade commodities in the Asian-Pacific region, particularly in countries such as Thailand, Malaysia and the Philippines (Tudkaew *et al.*, 2008). In spite of intensive marine cage culture in the last decade, the main market was secured by marine reserves harvest fish through trawl net fishing (FAO/NACA, 2000). Young fish are usually found in estuaries and silty areas, although they have also been observed in freshwaters. Adults are often found in marine offshore areas at depths of about 100 m and have only been reported in a few parasitic invasion in *Epinephelus coioides*, such as kidney sphaerosporosis and some parasitic helminthes (Supamattaya *et al.*, 1990; Cribb *et al.*, 2002). Epidermal parasitic and some bacterial infections in grouper have also been observed (Chong and Chao, 1984). Abdul-Salam *et al.*

(1990) have reported a trematode species (Didymozoidae: *Gonapodasmius epinepheli*) from an *Epinephelus tauvina* in the Persian Gulf. Abdul-Salam *et al.* (1990) described the primal taxonomic characteristics of the parasite, for instance, a bulbous bulged at the distal end of the ovary, prevalence of a swelling in the genital junction region and a dense bulk of glandular cells around the limbs of the intestine, and eventually described it as a new species. In the present study host reaction and pathogenicity of *Gonapodasmius epinepheli* from an *Epinephelus coioides* in the coastal area of Khark Island, adjacent to Bushehr province, in the Persian Gulf, Iran was discussed.

**Materials and methods**

Twenty five grouper fingerlings (158.54 ± 18.22 g and 412 ± 22.43 mm) were collected (October-December 2009) from cages in the southeast coast of Khark Island and transported to Microbiology laboratory of Iran Shrimp Research Center. Cysts of parasites in different sizes and color (white and yellow) from primary gill lamellae were measured under dissecting microscope (Nikon SMZ1000,

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japan) and parasites were isolated and identified under the light microscope (Nikon Photolab, Japan). The gill filaments from newly killed fish were ablated, then fixed and dehydrated in 10% buffered formalin and alcohol, respectively. Dehydrated gills embedded in wax, then sectioned at 5  $\mu$ m and stained with hematoxylin and eosin. Some capsules were dissected with a needle to release live parasites. Identification of parasites was carried out using the key to diagnose digenetic trematodes as delineated by Yamaguti (1971). Afterward they were dyed with azocarmine, and permanent histopathological slides were prepared. Eggs and adult parasites were assessed under an ocular mi-

crometer.

## Results

The diagnosis of *Gonapodasmius epinepheli* was performed According to Abdul-Salam *et al.* (1990) description such as a bulbous swelling at the distal end of the ovary, prevalence of a bulge in the genital junction region and a dense mass of glandular cells around the intestinal limbs.

The cysts were observed on the filaments of the first gill arch of the right and left gill, or both (Fig. 1A). The opaque white and yellow cysts were tube-

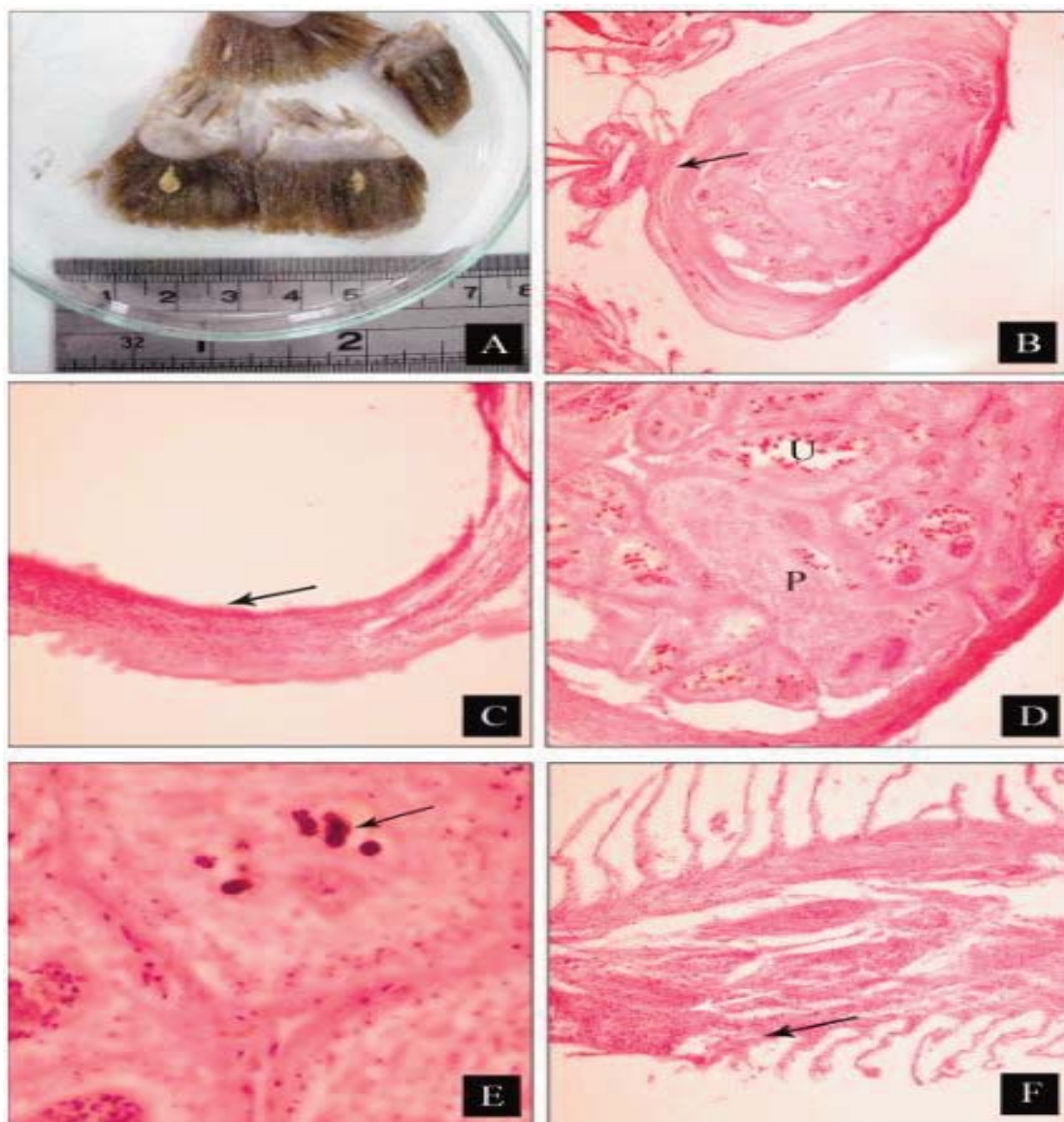


Fig. 1. (A) Separated gill arch showing parasitic cysts of *Gonapodasmius epinepheli* (in different size) on the gill lamellae, hematoxylin and eosin staining. (B) Cyst found attached to the primary lamellae  $\times 10$  (C) Cyst walls composed to host tissue; (D) the cyst including uterine (U) of parasite (P)  $\times 20$ . (E) Eggs within the uterus lumen  $\times 40$ , stained with azocarmine dye. (F) Lymphocytic infiltration into the area of infestation (white arrow), area of infection showing the reduction of secondary lamellae and mild hyperplasia of the interlamellar epithelium occurred in the infected filaments (black arrow)  $\times 10$ , hematoxylin and eosin staining.

like or egg-shaped and were longitudinally connected along the posterior surface of gill filaments. Six of the 25 groupers were found to be infected and had one or two yellowish, tube-like to egg-shaped capsules on the first gill arch of the right and left gill. The cysts were also connected lengthwise along the posterior surface of the gill filaments. Depending on the host size, various cysts in size were observed. The white cysts measured  $4.0 \times 2.5 \times 1.0$  mm while the yellow cysts measured  $3.0 - 6.5 \times 2 - 4.0 \times 1.0 - 2.0$  mm. Isolated worms showed a variation in color according to the sex. Female worms were yellowish and male worms were more whitish. These differences were related to the color of eggs inside their bodies. One female and One to three males were collectively observed in each cyst. Female worms were commonly longer and larger than males. Capsule eggs were detected in the female uterus with a size range of  $17.07 \pm 1.21$  mm  $\times$   $11.55 \pm 0.92$  mm. In Fig. 1E, an operculum-like of egg was observed. According to the site of infection, as well as the type of host it, can be suggested that this parasite is the selfsame to *Gonapodasmius epinepheli* described by Abdul-

Salam et al. (1990).

Gills were the main site of infection preponderantly on the lower gill arch (Fig. 1A). Epithelial cells of primary gill lamellae and overlying several layers of connective tissue were observed in cysts wall (Fig. 1B). In histopathological sections, parasites were observed beneath the mucosal layer of the primary gill lamellae, and secondary lamellae had completely disappeared. No parts of the cyst wall comprised parasite tissue (Fig. 1C). Fig. 1D show cyst, including uterine of parasite in the histological section. In involved areas, parasitic cysts blew up and destructed secondary lamellae (Fig. 1F). Involved gill filaments were contorted in shape and contained several sections of the worms curled circa each other (Fig. 1F). The parasites were often partly or completely destroyed by themselves. Some host reactions were also observed in the surrounding area, like proliferation of capillaries on the cyst wall and infiltrations of lymphocytes (Fig. 1F). Sometime, only the parts of parasite cuticles or egg shells were observed in the cysts, and most organs had destroyed (Fig. 2A).

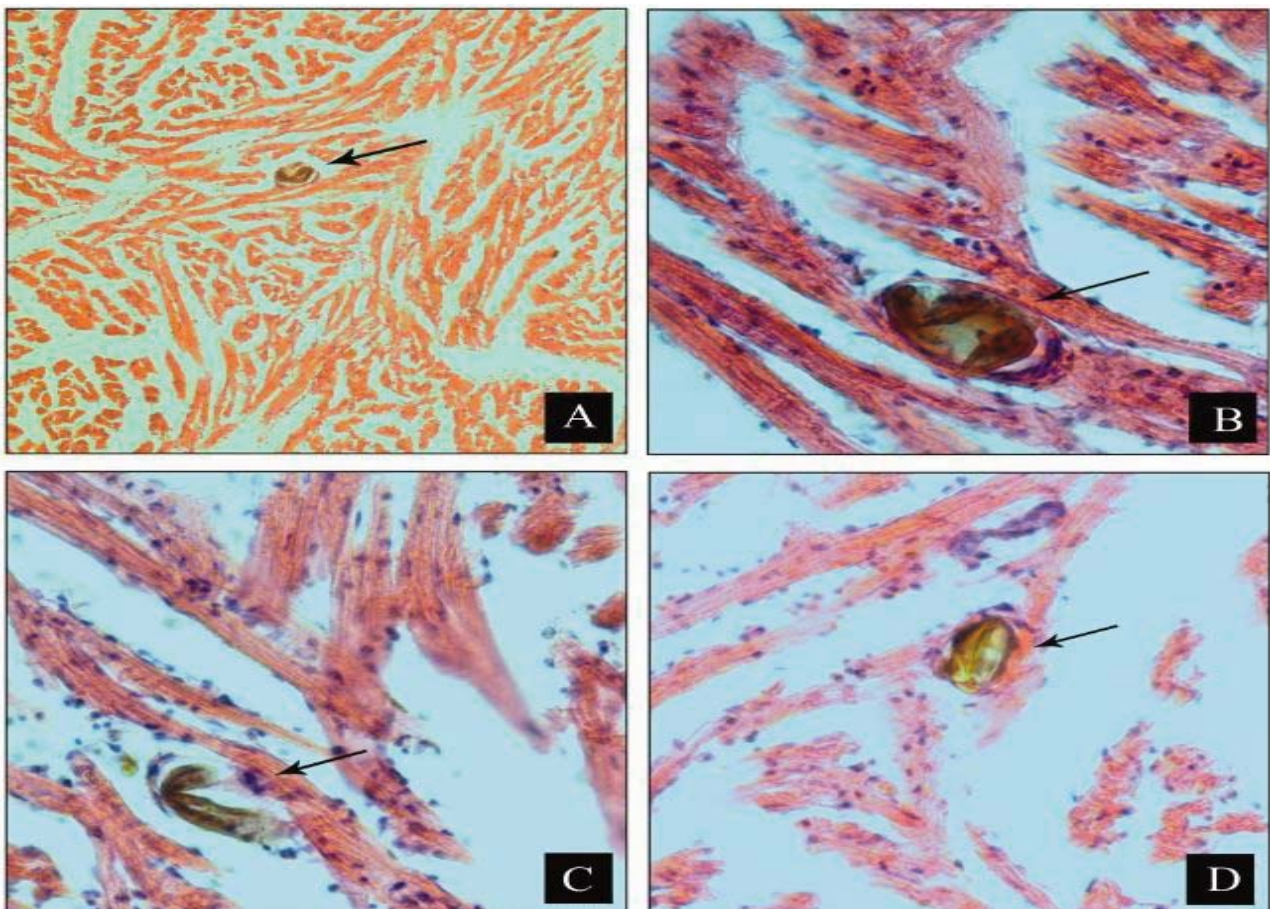


Fig. 2. *Gonapodasmius epinepheli*. (A) Parasite egg shells are encapsulated,  $\times 10$  and (B, C) engulfed egg shells by endocardial cells (arrow indicating layer of fibrocyts),  $\times 40$ . (D) Eggs of parasite are engulfed by endocardium of the ventricle  $\times 40$ , stained with azocarmine dye.

## Discussion

This is the first record of a didymozoid from the *Epinephelus coioides* in Khark Island, in the Persian Gulf, Iran. Didymozoids are frequent in many tropical pelagic fishes but in some benthic fishes such as platycephalids and serranids were reported. Invasions by didymozoid trematodes (*Gonapodasmius sp.*) have been observed in many species of grouper generally in gill arches, buckle cavity and skin (Yamaguti, 1971), but only a few studies have reported trematode infection from southwest Asia. The first report of *Gonapodasmius epinepheli* from Iran was recorded by Derakhshanfar and Radfar (2002), in *Epinephelus tauvina* caught from Khozestan coast in local journal. In our study, the life cycle of this parasite and its histopathogenesis were discussed. *Epinephelus coioides* is nearly related to *Epinephelus tauvina* and this leads to confusion in frequent evaluation (Allen, 1997). Five didymozoids contain *Allonematobothrium epinepheli* (Yamaguti, 1965); *Allonematobothrium xishaense* (Lester, 1980), *Indoglomeritrema epinepheli* (Madhavi and Hanumantha Rao, 1983), *Gonapodasmius pacificus* (Yamaguti, 1938) and *Gonapodasmius epinepheli* (Abdul-Salam et al., 1990) have been reported from *Epinephelus tauvina*. The first two come about on the fins, operculum or gill arch, but not on gill filaments as in our samples, and differ in other signs. In cases that occur on the filaments such as, *Indoglomeritrema epinepheli* is hermaphroditic, has a much smaller oral sucker and only a primarily pharynx. *Gonapodasmius pacificus* is more complicated and dimorphic, has a much larger oral sucker, pharynx and acetabulum. Observed parasites in this study most nearly resemble *Gonapodasmius epinepheli*, described from *Epinephelus tauvina* by Abdul-Salam (1990). Identification of didymozoids will be facilitated by molecular analysis (Anderson and Barker, 1993). A review of the biology and pathology of didymozoids has been given by Woo 2006. Didymozoids are normally dimorphic marine parasites (Yamaguti, 1970). These parasites can be found in capsules of connective tissue in some organs such as fins, skin, mouth cavity, operculum, muscles, stomach, intestine or liver (Lester, 1980). In our study, didymozoids were found between the epithelium basal membrane and efferent artery of the master gill filament. The responses of the host were limited only to the infected filaments. A deforma-

tion in the shape of the filament, slight hyperplasia of the interlamellar epithelium, (Madhavi and Hanumantha Rao, 1983), and a chronic inflammation was observed by cellular infiltration and fibrosis as a response of *Epinephelus tauvina* to the gill didymozoids, *Indoglomeritrema epinepheli* and *Gonapodasmius epinepheli*, respectively as described by Abdul-Salam and Sreelatha (1992). Moderate hyperplasia of the interlamellar epithelium and reduction of secondary lamellae were observed in the involved filaments (Fig. 1F). Hyperplasia of the gill epithelium has been reported in some fish invaded with parasites (Cruz-Lacierda et al., 2001). According to findings, the parasites stimulate only a local response. It must be considered that, long duration. infections, may be associated with more extensive damage. Eggs were observed in small blood lacunae of gills or lymphatic vessels of heart tissue (Fig. 2D). These eggs showed empty or degenerated egg shells (Fig. 2B and Fig. 2C). Host responses and lymphocytic reactions were slight in heart and gill tissues. In the head kidney, eggs were chiefly reported inside macrophages but sometimes seen in sinuses (Tudkaew et al., 2008). In fact, all destroyed eggs led us to the conclusion that the distribution of eggs cannot establish a reinfection. In a typical life cycle of most digenetic trematode, the eggs must be released into the surrounding water to find an intermediate host. Some of the eggs can be shed from mature worm to the blood circulation and distributed by the blood stream. The mortality in involved fish may have been associated with the invasion of monogeneans rather than the didymozoids. Cruz-Lacierda et al. (2001) recommended formalin bath as an effective treatment for controlling the monogeneans.

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