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*Original article*

# Histopathology of the *Haplorchis taichui* infection in the freshwater fish, Tigris kingfish, and Tigris barb (Cypriniformes: Cyprinidae) from Iran

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## Abstract

Flukes can cause severe and lethal diseases in various animals, including fish. Both adult and larval stages of flukes are found in fish. Haplorchiasis is an infection of fish gills by heterophyid trematodes such as *Haplorchis taichui*. To detect this parasite, the gills of 30 Tigris kingfish and Tigris barb collected from the Shapour River in Kazerun, Fars province were found to be parasitized with metacercarial cysts of a heterophyid trematode identified as *H. taichui*. Histopathological examination of the infected fish gills revealed cartilage proliferation, severe hyperplasia, fusion, S-forming, shortening and thickening, distortion, and displacement of affected secondary gill filaments leading to deformities of the filament structure, clubbing, telangiectasis, and hyperemia. Although the gill damage was evident and potentially life-threatening for the cyprinid fish, the examined fish showed no clinical signs. This finding indicates that *H. taichui* is pathogenic; therefore, prevention of infection and treatment should bea priority.

**Keywords:** fish-borne trematodes, histopathology, Kazerun, metacercariae, Shapour River

## Introduction

Metacercariae can impact the growth and survival of fish or cause disfigurement, resulting in economic losses (Leatherland 2006). The intestinal trematode, *Haplorchis taichui* (Nishigori 1924), is significant in public health as it causes infections in humans and animals, particularly in Asia, Africa, and the Americas (Chai et al. 2005, Dung et al. 2007). *H. taichui* is the most prevalent species among intestinal flukes in Eastern Asia (Wongsawad et al. 2009). Currently, this parasite is widespread in Taiwan, the Philippines, Bangladesh, India, Sri Lanka, Palestine, Iraq, Egypt, Malaysia, Thailand, Laos, Vietnam, and South China (Africa et al. 1940, Velasquez 1982, Yu and Mott 1994, Belizario et al. 2004, Chai 2007, Dung et al. 2007, Chai and Jung 2017). Recently, Golchin Manshadi (2021) reported the presence of *H. taichui* in Iran. The highest occurrence of heterophyid metacercariae was observed in the muscles of the caudal region of various fish species. Kumchoo and Chai (2023) found metacercariae of *H. taichui* and *H. mehrai* in the muscle, fin, and scale of fish. Adult heterophyids reside deeply embedded in the intestinal mucosa of mammals and birds, where they produce fully embryonated eggs excreted in feces (Sithithaworn et al. 2014). Human infection can occur through the consumption of raw or improperly cooked cyprinid fish containing metacercariae (Kumchoo et al. 2005). Teimoori et al. (2019) reported the infection of a jackal (*Canis aureus*) with the fish-borne trematode, *Haplorchis taichui* (Trematoda: Heterophyidae) in southwestern Iran (Khuzestan province). In the same area, metacercariae of *H. taichui* and other heterophyids, *H. pumilio*, *Stellantchasmus falcatus*, and *Centrocestus formosanus* were detected in various fish species (Farahnak and Massoud 1999, Farahnak et al. 2006). Approximately 100 species of snails have been identified as first intermediate hosts for trematodes, including *H. taichui* (Caron et al. 2008). *Melanoides tuberculata*, a tropical freshwater gastropod native to eastern Africa and the Middle East, has widely established itself throughout tropical regions. Pointier et al. (1992) demonstrated the presence of *M. tuberculata* in all intertropical continental countries and most Pacific islands. Cyprinid fish have been identified as important second intermediate hosts of heterophyid trematodes (Srisawangwong et al. 1997, Sukontason et al. 1999, Wongsawad et al. 2009). The fish population in the Shapour River (located at 51°31′771″ S and 30°7′542″ W) in Kazerun, Fars province, which spans 220 km, plays a significant role in the region's agriculture and water supply economy. While no heterophyid infections have been reported in this region to date, *M. tuberculata* has been found naturally infected by the parasite in the Shapour River (Golchin Manshadi and Mirghafari, 2015), creating a conducive environment for the maintenance of the life cycle of heterophyid trematodes such as *H. taichui*. Given the limited reports of heterophyid infections in Iran, it is imperative to continue and expand this research to other regions of the country.

## Materials and Methods

### Animals and study site

The study involved the collection of thirty live Tigris kingfish (*Cyprinion macrostomus*) and Tigris barb (*Capoeta barroisi*) from the Shapour River. The fish were captured using gill nets or angling by local fishermen and transported live to the laboratory in aerated tanks.

### Sampling procedures and experimental design

Upon arrival, the fish were euthanized by decapitation, and their gills were examined under a stereomicroscope. The gills were carefully removed, placed on glass slides with saline solution, and examined using optical microscopy to identify and count any *H. taichui* metacercariae present. The isolated parasite was confirmed as *H. taichui* through morphological and molecular analysis. For histopathological examination, some affected gill arches were dissected, fixed in 10% buffered formalin, embedded in paraffin wax, and sectioned. The tissue sections were dehydrated using a series of ethanol solutions, and then stained with hematoxylin and eosin (H&E) for microscopic analysis. The study was conducted as part of a project approved by the Research Ethics Committee on Animal Ethics at the Islamic Azad University, Kazerun Branch, Iran (IR.IAU.RES. No: 1398.150).

### Statistical analysis

The collected data were analyzed using IBM SPSS Statistics version 20 to determine the mean abundance and mean intensity of infection. This statistical analysis would provide insights into the prevalence and severity of the *H. taichui* infection in the studied fish species.

## Results

The examination of gill specimens revealed that 13 out of the 30 fish examined (43.33%) were infected with encysted metacercariae of *H. taichui*, with 9 Tigris kingfish and 4 Tigris barb showing infections. The mean intensity of encysted metacercariae (EMC) was

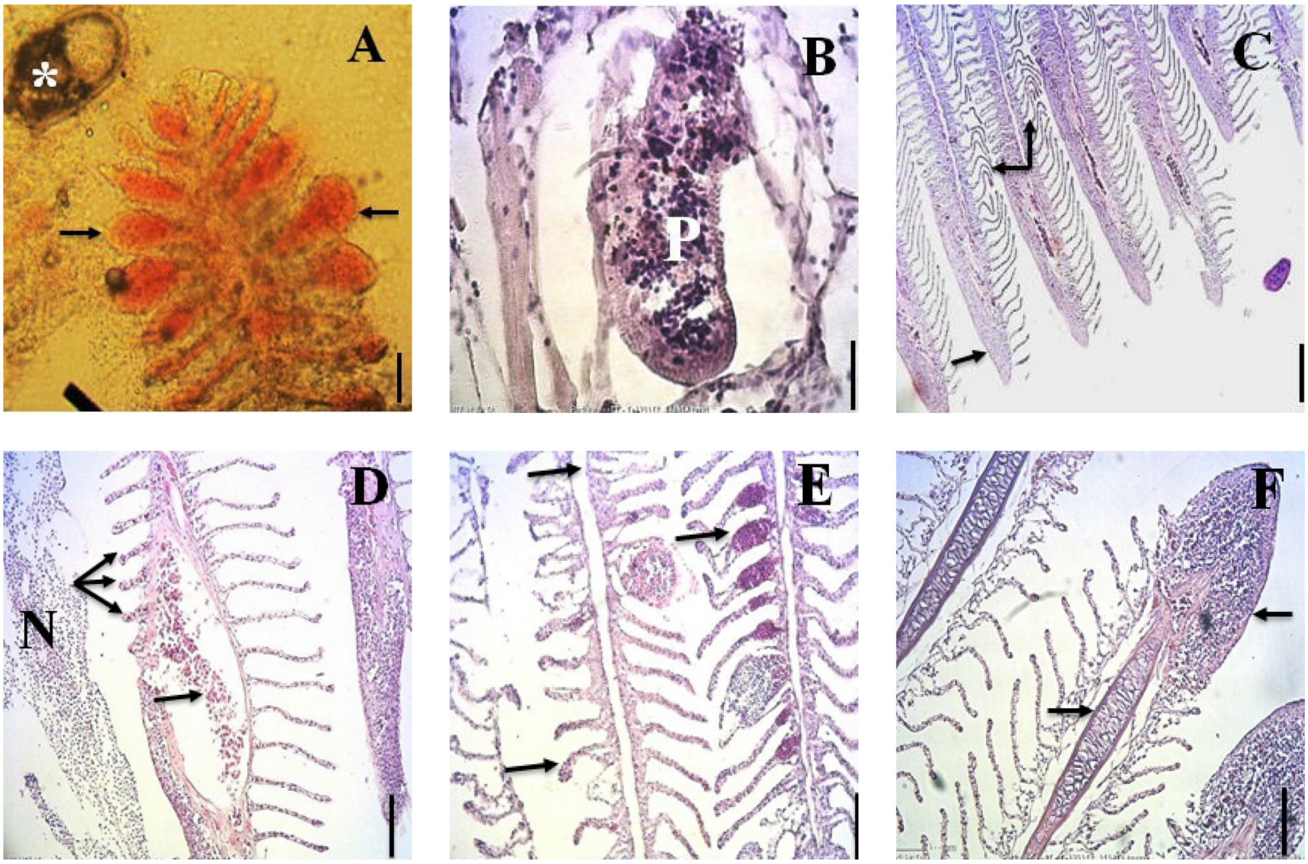


Fig.1. Histological section of infected gills of *C. macrostomus* and *C. barroisi* infected to *H. taichui* : A. Telangiectasia, the reversible swollen blood vessels in the secondary gill lamellae (arrows), encysted metacercariae (white star), wet mount, (scale bar = 50 µm). B. Encysted metacercariae (P), Hematoxylin and Eosin staining, (scale bar = 20 µm). C. Deformity (S- forming) of secondary gill filaments structure (up arrows), hyperplasia and fusion in the affected secondary gill filaments (down arrows), H & E, (scale bar = 200 µm). D. Necrosis of gill filaments (N), thickening and shortening of secondary gill filaments (up arrows), hyperemia (down arrow), H&E, (scale bar =100 µm). E: separation of gill filaments structure (up arrow) Telangiectasia (middle arrow) and starting of clubbing gill filaments (down arrow), H & E, (scale bar = 100 µm). F: Severe hyperplasia of distal secondary filament (up arrow), cartilage proliferation (down arrow), H & E, (scale bar = 100 µm)

3.58±23.99 cysts per fish, with a range of 1 to 15 cysts per fish. Specifically, Tigris kingfish had a mean EMC of 3.72±69.29, while Tigris barb had a mean EMC of 2.86±72.33. Although the infected fish did not display specific clinical signs, examination of wet-mounted gills revealed swollen and deformed branchial tissues. A distinct tissue layer surrounding the cysts indicated a host reaction (Fig. 1 A). Telangiectasia in the secondary lamellae and congestion on the gill filaments were also observed during wet mount and histopathological examination (Fig. 1 A and E). The histopathological results corroborated the wet mount observations, providing additional details. Infected gill filaments exhibited shortening, thickening, distortion, and areas of necrosis (Fig. 1D and E). Epithelial hyperplasia and fusion of filaments were also observed (Fig. 1 C and F). Engorgement with red blood cells, cartilage lesions, and epithelial hyperplasia leading to the disruption of normal gill morphology were noted in affected lamellae (Fig. 1 D). The proliferation of cartilage and epithelial tissue resulted in the modification of gill lamellae structure, fusion of lamellae, and loss of respiratory surface in some areas. The extensive epithelial hyperplasia formed “S”-shaped filaments, significantly altering the normal gill morphology (Fig. 1 C and F). Table 1 provides a detailed description of the numbers, prevalence, and mean ± standard deviation of gill lesions in infected fish.

## Discussion

A few trematodes cause harm to their fish hosts. Migration of cercariae through host tissues may produce minimal mechanical damage and hemorrhage (Hoffman 1999) and may also modify host behavior to increase the chance of being eaten by a definitive vertebrate host (Overstreet and Curran 2004). Some findings showed a high prevalence of metacercariae infection in fish. For instance, Wongsawad et al. (2011) found *H. taichui* and *Haplorchoides* sp. metacercariae in three species of cyprinoids had the highest preva-

Table 1. Numbers, frequency percent and mean ± standard deviation of gill lesions in infected Tigris kingfish and Tigris barb to metacercariae of *Haplorchis taichui*

| Gill Lesions | Tigris kingfish (9 fish) N(P%) | Tigris barb (4 Fish) N(P%) | Total (13 Fish) N(M ± SD) |
|---|---|---|---|
| Cartilage proliferation of primary filaments | 7 (77.77) | 3 (75) | 10 (76.38±9.07) |
| Severe hyperplasia in the basal, middle and apical portion of the gills ‘filaments | 9 (100) | 3 (75) | 12 (87.5±26.55) |
| Fusion of primary and secondary gill filaments | 5 (55.55) | 1 (25) | 6 (40.27±95.63) |
| S-forming of secondary filaments | 2 (22.22) | 1 (25) | 3 (23.61±11.36) |
| Shortening and thickening of secondary filaments | 7 (77.77) | 4 (100) | 11(88.88±31.92) |
| Distortion and displacement of gill lamellae | 6 (66.66) | 2 (50) | 8 (58.33±13.54) |
| Clubbing in the apical portion of secondary gill lamellae | 3 (33.33) | 1 (25) | 4 (29.16±10.61) |
| Telangiectasis in the base of the secondary gill lamellae | 3 (33.33) | 1 (25) | 4 (29.16±10.61) |
| Hyperemia of gill lamellae | 2 (22.22) | 0 (0) | 2(11.11±131.72) |
| Epithelial necrosis of gill lamellae | 6 (66.66) | 3 (75) | 9 (70.83±16.67) |

N – Number, P – Prevalence, M±SD – Mean ± Standard Deviation

lence (100%) in Chiang Mai, Thailand. Nithikathkul and Wongsawad (2008) found the prevalence of *H. taichui* and metacercariae in fish of the Mae Ngad and the Mae Kuang Udomtara Reservoirs to be 83.9% (26 out of 31 fish). Sukontason et al. (1999) reported that among the fish collected, *Puntius leiacanthus* contained the highest number of metacercariae of *H. taichui* (182 metacercariae per fish). Srisawangwong et al. (1997) showed that among four species, *H. taichui* was predominant and found in all kinds of fish examined. In the current study, the prevalence of *H. taichui* metacercariae stood at 43.33%, exhibiting notable variance from the studies cited, and the mean intensity of EMC was not high (3.58±23.99). Lesions of the gills associated with parasitic infection vary with the agent, host, and density of infection (Roberts 2001). These parasites have implications for fish welfare, the environment, and human health, necessitating prevention of their introduction into non-endemic regions. Occasionally, heavy trematode infections in fish can result in serious tissue displacement, secondary bacterial infection, and death (Overstreet and Curran 2004). In the present study, the fish showed no clinical symptoms, which may be due to low infection at the metacercarial stage of the parasite and the natural, unstressed conditions of the fish living in the river. In such conditions, pathogenic agents cannot dominate the sensitive host. Mitchell et al. (2000) suggested that when gill damage is severe and metacercarial infection in the fish exceeds 800 cysts per fish, life-threatening or fatal outcomes may occur. In comparison, the mean intensity of EMC in this study was 3.58 ± 23.99. Reports of metacercariae causing the proliferation of gill cartilage have all been putatively caused by members of the family Heterophyidae (Olson and Pierce 1997). Histopathological observations of infected gills in some studies have shown that parasite metacercariae lodged next to the filaments’ cartilage cause an intense inflammatory response, including hyperemia, hyperplasia of the cartilage of the primary lamellae enveloping metacercarial cysts, fusion of secondary lamellae, proliferation of fibroblasts, and chondroblasts (Mood et al. 2010). In several studies, extensive cartilage proliferation was observed, often leading to the fusion of gill filaments. The proliferated cartilage caused filaments to expand significantly, disrupting the normal gill morphology. Filaments appeared thick, distorted, and fused, with few typical lamellae present. Epithelial hyperplasia was extensive in these cases (Olson and Pierce 1997, Blazer and Gratzek 1985, Mitchell et al. 2000, Gjurcevic et al. 2007, Walaa et al. 2019). In the current study, while cartilage proliferation did not significantly alter the gill lamellae structure in most cases, hyperplasia of the filament epithelium did lead to fusion of gill lamellae in some instances. However, the thickening, shortening, distortion, and deformity of secondary gill filaments were considered insignificant. Telangiectasia and clubbing were not documented in similar research, except for telangiectasia of branchial blood capillaries in the study of the impact of prevalent parasitic diseases on pathological alterations in African catfish (Awadin et al. 2012) and congestion in the central venous sinus of the primary lamellae (Walaa et al. 2019). In the present study, mild telangiectasis was identified at the base of secondary gill filaments, and clubbing was observed at the apical portion of the secondary gill filaments in certain specimens. Walaa et al. (2019) noted primary and secondary lamellae necrosis of the gill in Nile tilapia due to metacercarial infection. This study also found necrosis of the primary and secondary lamellae in limited areas, especially next to metacercarial cysts. Mood et al. (2010) reported an intensive inflammatory response, including hyperemia and hyperplasia of the cartilage of the primary lamellae. Awadin et al. (2012) demonstrated atrophy and degeneration of central blood sinuses and hemorrhages in branchial blood capillaries. Affected lamellae often exhibited areas engorged with red blood cells (Mitchell et al. 2000). Hyperemia was observed in the central venous sinus in limited cases in the current study. Gill infection can result in structural and functional damage to the gills, leading to lower tolerance to hypoxia (Santiago Bass et al. 2007). Observations of infected fish suggested that while parasites may cause cartilage proliferation, the resulting damage to the respiratory surface of the gills may not be sufficient to directly cause mortality, as confirmed by the findings of Olson and Pierce (1997) in steelhead trout. When parasites interact with other stressors, the most obvious interaction is that some stressors may make hosts more susceptible to parasitism and exposure to unfavorable conditions, such as environmental factors, which can increase parasite infection rates (Kuris 1997). Therefore, it appears that fish mortality due to parasitic infection in fish farms exceeds that in other environments, such as rivers, lakes, and seas.

Finally, it is important to note that the prevalence of zoonotic trematodes is very low in Iran, not only due to low fish consumption but primarily because of the good cooking habits of the people in this country. However, these trematodes can complete their life cycle in other reservoir hosts; therefore, they remain in the environment for longer. Thus, it is advisable to screen for trematodes in reservoir and intermediate hosts. Control strategies should include training traders and farmers to monitor the health condition of imported fish. However, the main concern for veterinarians and traders should be biosecurity measures, such as border inspections and fish quarantine measures. Additionally, appropriate legislation requiring health certificates showing the health status of imported fish batches is needed in the surveyed infected areas (Ditrich et al. 1992).

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