

Research Article



The Presence and Histopathology of Encysted Metacercaria of *Clinostomum complanatum* (Rudolphi, 1819) (Digenea:clinostomidae) in *Garra rufa* (Heckel, 1843)

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Abstract | The study describes the intensity and histopathology of the *Clinostomum complanatum* infection in *Garra rufa* used in the health and beauty industries in foot spas for ichthyotherapy. In total of 25 examined fish specimens 56% were infected with the metacercariae of *C. complanatum*. The mean intensity of the infection was 27.14 cysts per host, varying between 1-75 cysts. The parasites were determined encysted in the base of the fins, muscle, inner wall of the operculum, gill arches, lips, upper jaw, body cavity and palate, forming small 2-3 mm diameter white-yellowish nodules, easy to detect in macroscopical observation. The highest prevalence of the metacercariae was in *C. complanatum* with 59.73% in gill tissue. The parasites were found encapsulated by a thin connective tissue each containing a single parasite in muscle. Metacercariae of *C. complanatum* were caused necrotic and fibrotic muscle tissues lesions in *Garra rufa*.

Keywords | *Clinostomum complanatum*, *Garra rufa*, Histopathology, Prevalance, Intensity

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INTRODUCTION

Garra rufa (Heckel, 1843) commonly known as doctor fish, are freshwater cyprinid species that naturally inhabit river basins, muddy streams, ponds and lakes in Eurasia. They are widely used in the health and beauty industries at the spa center for ichthyotherapy. The spread areas of *G. rufa* were reported in Iraq, Iran, some coastal rivers in southern Turkey and northern Syria. *G. rufa* might poses a potential risk to customers or ornamental and native fish with zoonotic pathogens. Zoonotic agents could be transmitted not only directly from affected fish, but also indirectly through water (Bhattacharya, 2016).

Yellow spot disease is a common fish parasitosis. This trematode has a very economic importance because of dam-

age to fish carcasses depending on white-yellowish nodules (Sutuli et al., 2014). In addition to *Clinostomum* spp. is a fish-borne zoonotic parasite responsible for Halzoun syndrome in humans who consume raw or undercooked freshwater fish that infected by metacercariae (Kifune et al., 2000; Park et al., 2009; Sutuli et al., 2014).

Adult forms of digenean family Clinostomidae Lühe, 1901 were found in the mouth, esophagus and pharynx of fish-eating birds, reptiles. The first intermediate hosts are freshwater snails, and the second definitive hosts are many species of fish (Kanev et al., 2002). They are known as yellow grubs due to their color and encyst in organs such as muscles, dermis and gills or in the body cavity (Olsen, 1974; Bullard and Overstreet, 2008).

Clinostomum complanatum is a cosmopolitan trematode and low host specificity. The metacercariae have been morphologically reported from fresh and brackish water fish species in Turkey (Çolak, 2013; Soylu, 2013, 2014; Ozturk and Ozer, 2015) and molecularly (Simsek et al., 2018). Due to risk of disease transmission to humans, the metacercariae of *C. complanatum* have zoonotic potential (Pati-mar et al., 2010).

Ichthyotherapy was adopted and commonly commercialized worldwide including Japan, Croatia, China, Netherlands, Malaysia, Singapore and Korea. In spite of these medical preferences, unfortunately there are a limited number of reports about the potential pathogens that might be carried by *G. rufa* (Majtán et al., 2012). The infestation of parasites particularly trematodes as a serious zoonotic and threat to health were reported (WHO, 1995). Therefore, this is the first study to determine the histopathological effect of *Clinostomum complanatum* in *G. rufa* fish.

MATERIALS AND METHODS

G. rufa (9.5 - 10 cm total body length) was obtained from local aquarist in Antalya. Twenty five of these fish were immediately transported alive to the fish disease laboratory in suitable containers, and fish were dissected carefully for observed the presence of encysted metacercariae into the all organs. They were observed under a stereozoom microscope (Leica S6) for larval digeneans. The attached cysts visible to the naked eye were photographed by the present authors. Identification of *C. complanatum* metacercariae (Rudolphi, 1819) (Digenea: Clinostomidae) was made according to (Bychovskaya and Pavlovskaya, 1962; Yamaguti, 1958, Dawes, 1968; Markevic, 1951; Matthews and Cribb, 1998; Simsek et al., 2018; Cagatay et al., 2022). Prevalence, mean intensity and abundance of infection were measured following Bush et al. (1997).

The tissue samples containing the encysted metacercariae were fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin wax to cut 5 µm thick sections using a rotatory microtome, for histology and stained with haematoxylin and eosin (H&E). The DPX mounted sections were observed under microscope and photographed.

RESULTS

In total, of 25 examined fish specimens 56% were infected with the metacercariae (yellow grubs) of *C. complanatum* including *G. rufa*. The mean intensity value of the infection was 27.14 cysts per host, varying between 1-75 cysts. The parasites were found encysted in the fins bases, muscle, inner wall of the operculum, lips, gill arches, palate, up-

per jaw and body cavity, forming small 2-3 mm diameter white-yellowish nodules. The operculum and mouth were the most common locations of the parasites than other tissue. In the fins, the parasites were frequent in dorsal, anal and pelvic fins, while only very rarely they were present in the caudal fin (Figure 1 A-F).

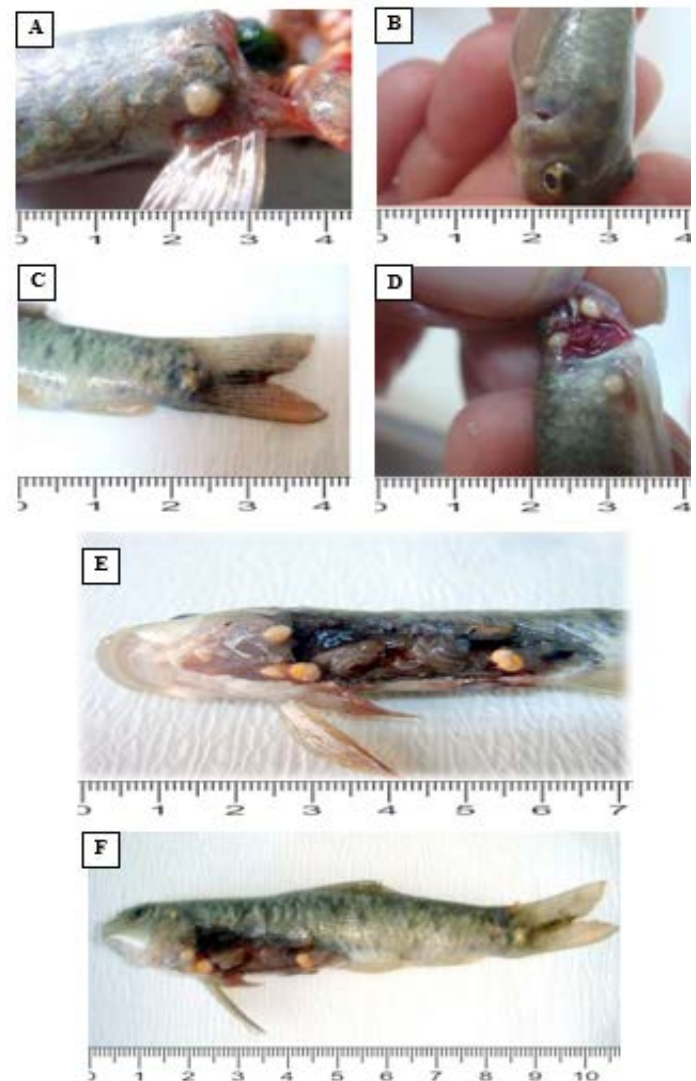


Figure 1: Metacercariae of *C. complanatum* (Rudolphi, 1819) on the base of fins (A, B, C), inner wall of the operculum (D) and body cavity (E, F) from *G. rufa*

The prevalence and intensity of parasites in *G. rufa* were 0.56 and 27.14, respectively. The metacercariae were observed as encysts and excysted worms in the muscles, buccal cavity, sub operculum and pectoral fins bases. The highest prevalence of the metacercariae was in *C. complanatum* with 59.73% in gill tissue (Figure 1D).

The prevalence, mean of intensity and abundance of parasites in different microhabitats were as follows: in the muscle (22.10%, 6 and 3.36), in the gill (59.73%, 16.21 and 9.08), in the different fins (3.94%, 1.07 and 0.6) and the other area of body (14.21%, 3.86 and 2.16) (Table 1).

Table 1: Infection intensity, prevalence, abundance on different microhabitats in *G. rufa*

Host Fish	Microhabitats in host fish	Total of parasites	Prevalance* (%)	Mean intensity**	Mean abundance***
<i>Gara rufa</i> (n=25) infected hosts=14	Muscle	84	22.10	6	3.36
	Gill	227	59.73	16.21	9.08
	Fin	15	3.94	1.07	0.6
	Other	54	14.21	3.86	2.16

*Prevalence: Number of parasites in per microhabitats divided by the number of parasites in total microhabitats expressed as a percentage.

**Mean intensity: Number of parasites in per microhabitats divided by the infected hosts examined.

***Abundance: Number of parasites in per microhabitats divided by the total fish examined.

The parasites were identified as *C. complanatum* metacercariae according to the described keys Matthews and Cribb (1998). The morphologic structures of *C. complanatum* metacercariae from the tissue of infected fishes were as follows: Oral collar visible. Ventral sucker larger than oral. Glandular structure present in forebody, anterior to ventral sucker. Intestinal caeca lateral to ventral sucker and genital complex, with prominent lateral pouches. Ovary irregular in shape, located dextrally in intertesticular space. Vitellaria not evident (Figure 2).

The histopathology of the infection of *G. rufa* by *C. complanatum* is described in this study. Gill lamellae surrounding the metacercariae were necrotic. Desquamation and thickness were seen in epithelium of gill tissue (Figure 3, 4). The parasites were determined encysted in the muscle (Figure 5). In all the locations the parasites were surrounded by a fibrous capsule consisting of a layer of dense connective tissue followed, in most of the cases, via a layer of loose connective tissue (Figures 5 and 6). The thickness of these layers varied with the location of the parasites. When the parasites encysted in the muscle tissue a slight degeneration of the muscle fibers was observed (Figure 6).

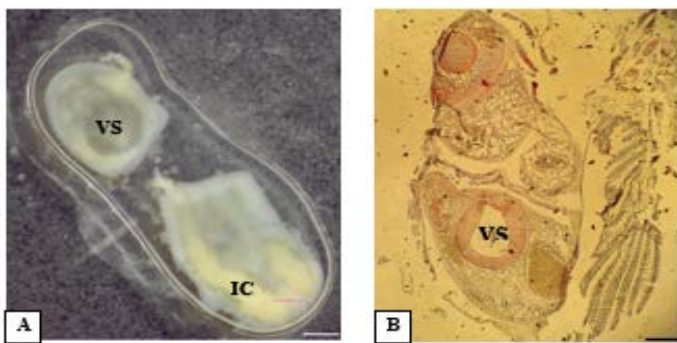


Figure 2: (A) The excysted metacercaria of *C. complanatum* (VS: ventral sucker; IC: intestinal caeca) (scale 500 µm) (B) *C. complanatum* metacercaria in histological section. Some microanatomical characteristics of the parasites (scale 50 µm)

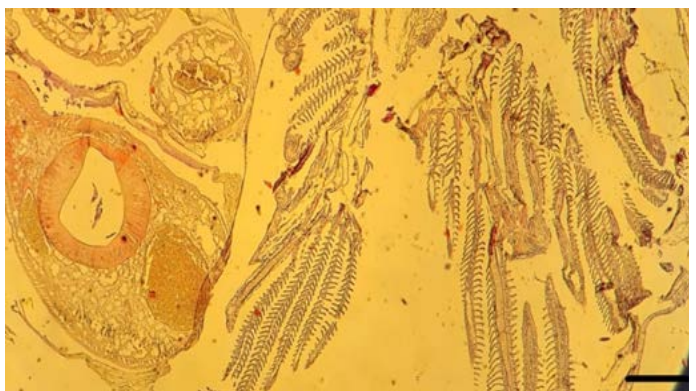


Figure 4: Severe tissue damage; thickness (thick arrow), desquamation (thin arrow), and hyperemia (star) of gill lamellae (scale 50 µm)



Figure 3: *C. complanatum* metacercaria (thick arrow) on the gill (arrow) (scale 400 µm)

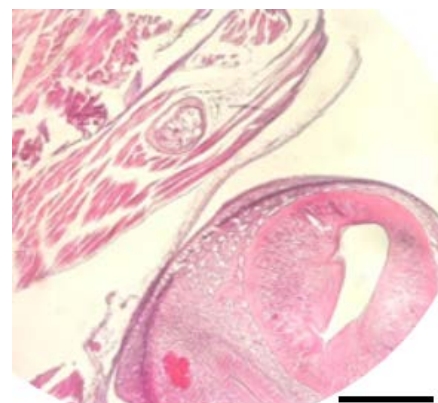


Figure 5: The encysted *C. complanatum* metacercaria surrounded by the cyst wall (thick arrow), the sucker of *C. complanatum* metacercaria (arrow) (scale 400 µm)

Table 2: Data from literature review studies about *Clinostomum* sp.

Fish Name	Species of Parasite	Country	References
<i>Danio rerio</i>	<i>Clinostomum</i> sp.	Brazil	Silveira <i>et al.</i> , 2021
<i>Trichogaster fasiatus</i>	<i>C. complanatum</i>	India	Rizvi <i>et al.</i> , 2020
<i>Garra rufa</i>	<i>C. complanatum</i>	Iranian	Meleki <i>et al.</i> , 2018
<i>Squalius cephalus</i>	<i>C. complanatum</i>	Turkey	Şimsek <i>et al.</i> , 2018
<i>Lepomis macrochirus</i>	<i>Clinostomum</i> sp.	United States of America	Calhoun <i>et al.</i> , 2018
<i>Myxocyprinus asiaticus</i>	<i>C. complanatum</i>	China	Li <i>et al.</i> , 2018
<i>Hemibarbs labeo</i>	<i>C. complanatum</i>	Taiwan	Wang <i>et al.</i> , 2017
<i>Synbranchus marmoratus</i>	<i>C. complanatum</i>	Brazil	Acosta <i>et al.</i> , 2016
<i>Hoplias malabricus</i>	<i>C. marginatum</i>	Brazil	Alcântara and Tavares-Dias, 2015
<i>Capoeta damascina</i>	<i>C. complanatum</i>	Israel	Caffara <i>et al.</i> , 2014
<i>Carassius carassius</i>	<i>C. schizothoraxi</i>	India	Shah <i>et al.</i> , 2013
<i>Oreochromis niloticus</i>	<i>C. tilapiae</i>	Kenya	Ochieng <i>et al.</i> , 2012
<i>Oreochromis niloticus</i>	<i>C. tilapiae</i>	Nigeria	Echi <i>et al.</i> , 2012
<i>Etheostoma nigrum</i>	<i>C. detruncatum</i>	Canada	Bonett <i>et al.</i> , 2011
<i>Rhamdia guatemalensis</i>	<i>Clinostomum</i> sp.	Mexican	Pérez Ponce de León <i>et al.</i> , 2009
<i>Lepomis macrochirus</i>	<i>C. marginatum</i>	North America	Zimmermann and Ingold., 2008
<i>Capoeta capoeta</i>	<i>C. complanatum</i>	Iranian	Malek and Mobedi, 2001
<i>Acheilognathus rhombea</i>	<i>C. complanatum</i>	Korea	Chung <i>et al.</i> , 1995
<i>Carassius carassius</i>	<i>C. complanatum</i>	Japan	Aohagi <i>et al.</i> , 1992
<i>Perca fluviatilis</i>	<i>C. complanatum</i>	Poland	Grabda-Kazubaska, 1974
<i>Alosa sapidissima</i>	<i>C. marginatum</i>	United States of America	Hollis and Coker, 1948

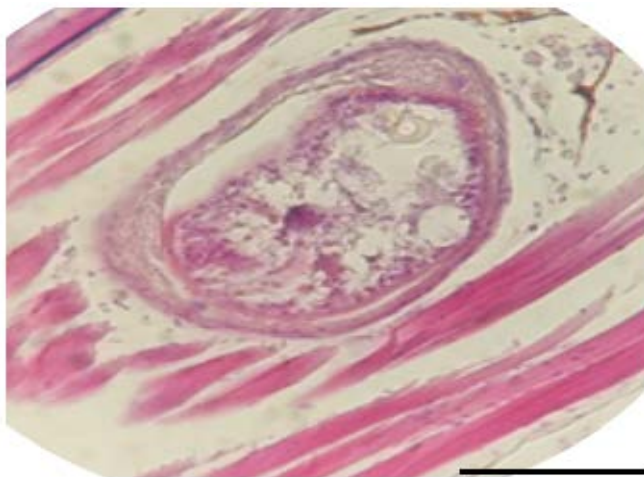


Figure 6: *C. complanatum* metacercaria encysted in the muscle, metacercaria cyst wall (arrow), necrotic and fibrotic muscle tissues surrounding the parasite (star) (scale 1000 µm)

DISCUSSION

Ichthyotherapy is becoming on increasingly popular for skin disorder. There are reports that *G. rufa* fish are helpful in the treatment of psoriasis and atopic dermatitis (Bhattacharya, 2016). These fish, feeding on dead skin on the body. However, there is a risk of transferring the zoonot-

ic agent from water to human. Freshwater fish species are known to harbour *C. complanatum* metacercaria (Table 2). They are embedded in the muscle or under the skin. The World Health Organization (WHO, 1995) has estimated that the number of people infected with fish-borne trematodes. Many cases of human infection of *C. complanatum* have been reported from various regions of the World (Hung *et al.*, 2013).

In this study, the prevalence and intensity of parasites in *G. rufa* were 56% and 27.14, respectively. The highest prevalence of the metacercariae was in *C. complanatum* with 59.73% in gill tissue. Also, Malek and Mobadi (2001) examined the *C. complanatum* in *Capoeta capoeta gracilis* from Shiroud River. They found the prevalence and abundance of parasites were significantly higher under the mouth to behind the opercule. The prevalence of infection was the highest in the 7-11 cm length group. In another study, Aohagi and Shibahara (1994) and Li *et al.* (2018) found that the main habitat of *C. complanatum* in *Carassius* spp. was the muscles around the gills and in the head region of *Monochamus asiaticus*, with lower levels of infection in the posterior part of the body, respectively. Maleki *et al.* (2018) determined the metacercariae (yellow grubs) of *C. complanatum* in the muscles, buccal cavity, sub operculum and pectoral fins bases in fish species.

The metacercariae of *C. complanatum* were known to cause considerable damage to the viscera and musculature of fish species (Menconi et al., 2020). The infected fish tissue revealed heavy infiltration of immune cells at the site of cyst wall (Shareef and Abidi, 2012). Lo et al. (1992) determined that when a large amount of cercariae of *C. complanatum* penetrate the fish (*Misgurnus anguillicaudatus*) body at the same time they may cause irritation and lesions to fish tissue, which may induce mortality of small fish. In this study, *C. complanatum* were found encysted in gill arches, fins, muscle, inner wall of the operculum and body cavity and metacercariae were caused necrotic and fibrotic muscle tissues lesions in *G. rufa*. These results are in agreement with the previous reports.

G. rufa are widely used in aesthetic practice such as fish pedicure. However, ichthyotherapy can be potentially dangerous procedure in immunocompromised persons (Pastorino et al., 2016). There is the a need for more studies assesing this procedure. The results of this study is supported the requirements of sanitary and legal aspects and pathogen screening procedure on the use of *G. rufa* for human health.

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CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

Trematodes, which constitute an important part of parasites, are zoonotic and pose a threat to consumer health. Therefore, our study is the first to determine the presence and histopathological effect of *Clinostomum complanatum* parasite in *G. rufa* fish. In this study, it was determined that it is very important to monitor *G. rufa* fish in terms of disease before using them in ichthyotherapy.

AUTHORS CONTRIBUTION

Conceptualization, Ö.Ö. and Ö.D.; methodology, Ö.Ö., Ö.D. and M.N.; investigation, Ö.Ö. and A.A.; resources, A.A.; writing-original draft preparation, Ö.Ö. and Ö.D.; writing-review and editing, Ö.Ö., Ö.D. and M.N. All authors have read and agreed to the published version of the manuscript.

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