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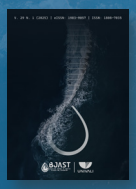

AN INVESTIGATION ON FINGERLING GRASS CARP *CTENOPHARYNGODON IDELLA* ECTOPARASITES WITH THE FIRST OCCURRENCE OF *TYLODELPHYS CLAVATA* AS A NEW LOCALITY RECORD IN NORTHERN IRAN

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ABSTRACT

Aquaculture in Iran has been developing quickly in recent years and includes the introduction of various new species such as Chinese carp (*Ctenopharyngodon idella, Hypophthalmichthys molitrix, and Hypophthalmichthys nobilis*), rainbow trout (*Oncorhynchus mykiss*) and stocks of common carp (*Cyprinus carpio*). The main aim of this study was to determine the external parasitic fauna of grass carp *Ctenopharyngodon idella*, from aquaculture facilities in northern Iran, alongside their prevalence level, intensity, and abundance. A total of 35 fingerlings of Grass carp with an average length of 10cm and an average weight of 7g was caught from a fish farm in Guilan province, northern Iran, and transported alive to the Parasitology laboratory in the autumn of the year 2019. Common procedures of Parasitology were performed for fish sampling. All specimens (prevalence of 100%) were infected with different parasite groups. *Diplostomum spathaceum* and *Dactylogyrus lamellatus* were respectively recovered from the eyes and gills of all fish, with a mean intensity of 14.8 and 8.6 each. Also, *Ichthyophthirius multifiliis* was observed at the highest level of 100% in the skin and gills, followed by *Gyrodactylus* sp. and *Tylodelphys clavata* were respectively detected in the skin and eyes of grass carp with the lowest prevalence value of 20% and intensity of 1, in common each. The record of *Tylodelphis clavata* in grass carp in the present study is considered the first report on the northern Iranian aquaculture system.

Keywords: Digenea. Eye. Prevalence. Host. Specimen. Aquaculture.

1 Introduction

Cyprinid fish include 45% of Iranian freshwater fish species (Coad, 1979). Aquaculture in Iran has developed rapidly in recent years, and includes the introduction of various new species such as Chinese carp (*Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, and *Hypophthalmichthys nobilis*), rainbow trout (*Oncorhynchus mykiss*) and stocks of common carp (*Cyprinus carpio*). Together with this introduction of new species and their associated parasites to Iranian aquaculture there has been translocation of species in the different fauna regions whereby the parasites are also transferred to different parts of the country.

Grass carp (*Ctenopharyngodon idella Valenciennes*, 1844) is an important fish species bred in cyprinid aquaculture facilities in Iran, and mainly cultured for both aquatic weed control and human consumption. This species originates from the Far East, particularly the Amur River, and was successfully bred in polyculture with common carp, silver carp, and bighead carp in Europe before being introduced to open waters (Dimovska and Stojanovski, 2020). Grass carp inhabits slow-flowing or non-running waters with lots of plankton and macro vegetation according to Stevanovski (2010). This fish is an herbivore that initially feeds on zoo and phytoplankton and later exclusively on aquatic plants.

Monogeneans can be significant pathogens of teleost under confinement, and their presence can produce chronic debility, unsightliness, poor growth and mortality of infested hosts (Thoney and Hargis, 1991). An important characteristic is that the fish parasitized by monogeneans, show necrosis and bleeding wounds in the integument and gills, serving as a gateway to different pathogenic protozoa and bacteria of complicated control (Flores-Crespo and Flores, 1993; Harris et al., 1998; Del Rio-Zaragoza et al., 2010). They are flatworms (Platyhelminthes), ectoparasitic which attach on their host's skin or gills by special attachment organs, posterior positioned (Park et al., 2007).

Pathogenicity of monogenean gill parasites from cultured fish was widely recognized by Paperna (1963) and Molnar (1972). *Dactylogyrosis* is a disease caused by the monogenetic trematode, *Dactylogyrus* sp. *Dactylogyrus* was found to cause severe damage to the gills of the highly affected fish. *D. lamellatus* is the most harmful parasite, and is now spreading throughout the country and causing high mortality in grass carp fingerlings (Jalali and Molnar, 1990). Adults of these parasites were found attached to the host's gill tissues by a characteristic structure having a row of hooks and sometimes suckers as well.

Žitnan et al. (1969) determined the presence of *Ichthyophthirius multifiliis* and *Dactylogyrus lamellatus* in *Ctenopharyngodon idella* from fish farms in Bosnia and Hercegovina. Kiškaroly et al. (1980) found that *D. lamellatus* is the most common parasite in grass carp from fish ponds with an occurrence of 13.03%. Bozorgnia et al. (2012) conducted parasitological experiments in a fish pond in Iran and observed the presence of *Dactylogyrus lamellatus* in *Ctenopharyngodon idella*.

Shamsi et al. (2009) identified *Dactylogyrus lamellatus* and *Dactylogyrus ctenopharyngodon* in *Ctenopharyngodon idella* of cyprinid fish ponds in several areas of Iran. Sengupta and Dalwani (2008) surveyed the parasitic fauna of *Ctenopharyngodon idella* in the Choghakhor lagoon, Iran and determined the presence of *Dactylogyrus lamellatus* on gills, metacercariae of *Diplostomum spathaceum* on eye lens and metacercariae of *Tylodelphys clavata* in corpus vitreous. Also, Barzegar et al. (2008) detected metacercariae of *Tylodelphys clavata* in corpus vitreous of *Ctenopharyngodon idella* in Iran.

Previously, little attention was paid to the eye parasites of fishes in Iran and the records were confined mostly to infection caused by *Diplostomum spathaceum* in the eyes of various freshwater fish species. However, several studies have recently been carried out on the parasites of eyes in fish inhabiting waters in the Zagros mountain area in western Iran. Therefore, our knowledge about the parasites of fish eyes has been promoted broadly, and many new species have been identified (Barzegar and Jalali, 2002, 2006; Jalali and Barzegar, 2005, 2006; Raeisi et al., 2006).

The present study was conducted to recover and identify prevalent parasitic fauna that infects the external body surface of pond-cultivated fingerlings grass carp in north Iran, with special attention to the eye`s metacercaria. Besides, their status in parasite communities such as the occurrence value, intensity, and, mean abundance of parasites was estimated.

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2 Materials and Methods

A total of 35 fingerlings of grass carp specimens with an average length of 10cm and an average weight of 7g was caught from a fish farm in Guilan province, northern Iran, and placed in aerated plastic container containing pond water and then transported immediately alive to the parasitology laboratory in the autumn of the year 2019. Upon arrival, the fish were anesthetized with clove oil (75 mg L^{-1}) and euthanized by spinal cord section, according to the guidelines and policies of the local and national animal welfare laws (Javahery et al., 2012 and Fernandes et al., 2017), then measured by length and weight individually.

At first, gross signs of parasites on fish bodies were examined externally, and lens of the eyes was removed from the socket and smashed between two slides with pressure second, live parasites were slightly compressed between a slide and a coverslip prior to being observed under a light microscope at a magnification of 4x to 40x. Skin biopsies were prepared for the entire length of the lateral body wall. A gill biopsy was collected from the second arch of specimens. Common procedures of parasitology for fish sampling were performed according to Stoskopf (1993).

Methods used for collecting, fixing and mounting of parasite specimens were as follows; Monogenea: Gills were cut out and placed in a petri dish. The vigorously moving monogeneans were removed from the gill filaments with a pipette, fixed in ammonium picrate and glycerol gelatin under a coverslip, then examined using a microscope at 4x to 10x magnification according to Fernando et al. (1972), and the extent of identification to the species level was based on the morphological characteristics of the copulatory organ and haptoral sclerites, such as anchors, bars, and hooks, as performed by Gusev (1985) and Bykhovskaya-Pavlovskaya et al. (1962). Monogenean species are distinguished by the morphology and the size of the sclerotised parts of the haptor and copulatory organ.

Digenea: metacercaria was collected in a 0.6% saline solution. The sample was placed with a little saline on a glass slide and appropriate pressure was applied, and then was fixed with 90% alcohol and washed in 70% alcohol and mounted in Canada balsam for permanent slide preparation.

Tylodelphys clavata was identified based on the morphological characteristics of its metacercariae. The identification was made using the keys provided by Gibson (1996), Kozicka and Niewiadomska (1960a). For identification of other parasite species we used the following keys; Bauer (1985, 1987) and Gussev (1983). The most successful preparations for every parasite species were photographed and are displayed. Classical epidemiological variables (prevalence, intensity and abundance) were calculated according to Bush et al. (1997).

3 Results and Discussion

According to the available data (table 1), all the 35 grass carp specimens with the prevalence of 100% and intensity of 181 were infected with different parasite species. The mean abundance was equal to the intensity, provided that the number of parasitized fish was the same as those examined. During the current study, *Diplostomum spathaceum* and *Dactylogyrus lamellatus* (Fig 2) were respectively recovered from the eyes and gills of these carp (table 2) with a prevalence value of 100%. Also, *Ichthyophthirius multifiliis* was observed at high levels in the skin and gills of total fish (100% infected), follows by *Gyrodactylus* sp. and *Tylodelphys clavata* (Fig 1) which were detected in the skin and eyes of grass carp respectively with the lowest prevalence value of 20% each (only seven fish infected) as shown in diagram 1.

Table 1. Infection of ectoparasite species in pond fingerling grass carp

parasite species	infected fish	prevalence	intensity	mean abundance	parasite range
Ichthyophthirius multifiliis (skin)	35	100	74 ± 16.95	-	52-94
Ichthyophthirius multifiliis (gill)	35	100	83.2 ± 15.35	-	67-100
Diplostomum spathaceum	35	100	14.8 ± 8.98	-	7-30
Dactylogyrus lamellatus	35	100	8.6 ± 3.20	-	5-13
Gyrodactylus sp.	7	20	1 ± 0	0.2 ± 0.44	0-1
Tylodelphys clavata	7	20	1 ± 0	0.2 ± 0.44	0-1

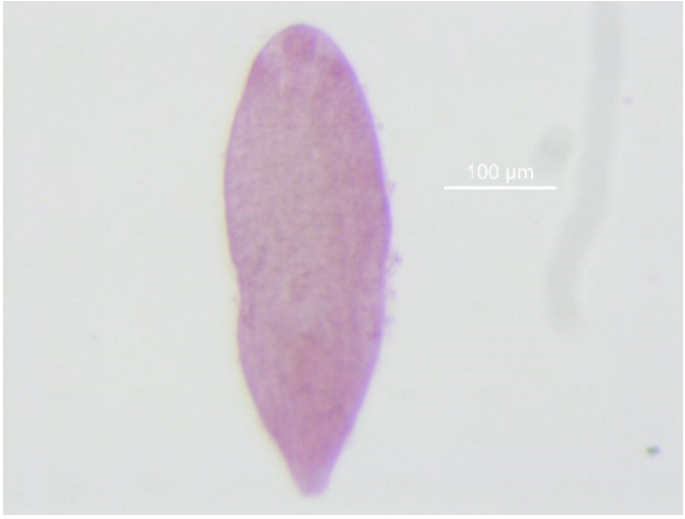

Figure 1. Tylodelphys clavata from eyes of grass carp-whole parasite. mag.40x

Table 2. Infection of ectoparasite species in pond fingerling grass carp based on collection site

Parasite species	infected fish	infection site	prevalence	intensity	mean abundance	parasite range
Ichthyophthirius multifiliis	35	Skin	100	74 ± 16.95	-	52-94
Ichthyophthirius multifiliis	35	Gill	100	83.2 ± 15.35	-	67-100
Ichthyophthirius multifiliis	35	Total	100	157.2 ± 32.08	-	119-194
Diplostomum spathaceum	35	Eye	100	14.8 ± 8.98	-	7-30
Dactylogyrus lamellatus	35	Gill	100	8.6 ± 3.20	-	5-13
Gyrodactylus sp.	7	Skin	20	1 ± 0	0.2 ± 0.44	0-1
Tylodelphys clavata	7	Eye	20	1 ± 0	0.2 ± 0.44	0-1

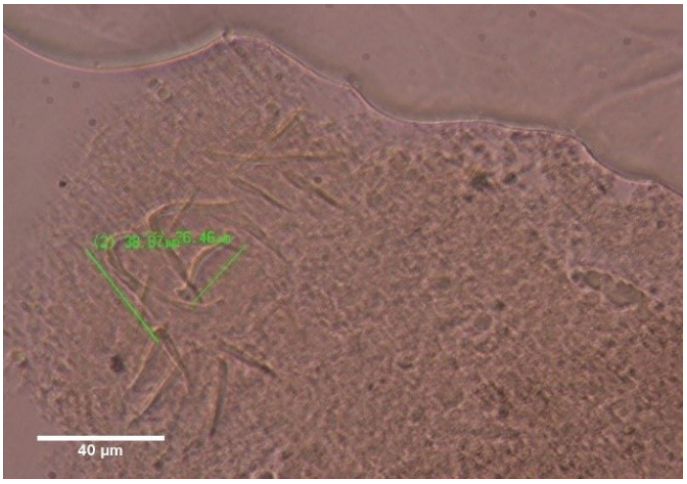

Figure 2. The attachment apparatus of Dactylogyrus lamellatus recovered from gills of grass carp. mag.40x

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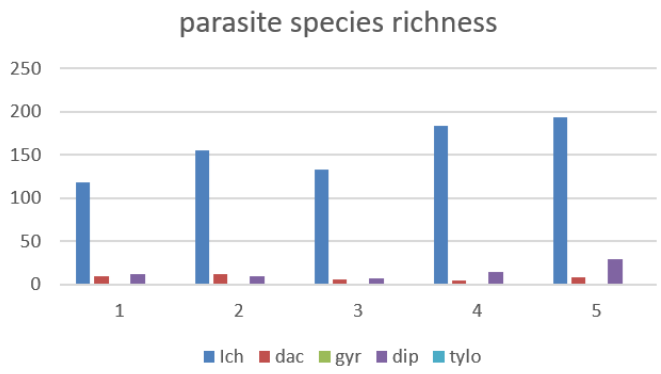

Diagram 1. Parasite species richness

The highest intensity of infection was observed in *Diplostomum spathaceum* (14.8) follows by *Dactylogyrus lamellatus* (8.6). The lowest intensity of parasites was found in both Gyrodactylus sp. and Tylodelphys clavata in common (1) (table 1). The lowest prevalence (23.68%) and mean intensity (4.78) in *Ctenopharyngodon idella* were previously established with *Tylodelphys clavata* from a Cyprinid aquaculture facility in N. Macedonia, which is a little different from our research. Moreover, grass carp was introduced as a new host for this parasite in Macedonian aquaculture (Dimovska and Stojanovski, 2020).

The prevalence value of *D. lamellatus* in that probe was 30.26%, which is far less, and the mean intensity was 19.00, which is far more than from our study. *Dactylogyrus* is one of the largest genera of parasitic helminthes of which 95% are parasites of gills in the fish of the family Cyprinidae considered by Dove and Ernst (1998). These species are well known for their high degree of host specificity (Jarkovsky et al., 2004). *Dactylogyrus* also has a direct life cycle which assists them to spread rapidly in the environment.

They are often found in lentic environments which promotes the transmission of these parasites (Flores-Crespo et al., 2003; Azevedo et al., 2007) and justifies that the fish had increased parasite infection since they are restricted to their culture ponds where water circulation is almost negligible or nonexistent. Under these conditions, *Dactylogyrus* can cause large bias and even death, as these parasites induce the gills to produce excessive mucus, making breathing difficult and consequently prejudice the fish's growth.

The prevalence value of presenting data except in the cases of *Gyrodactylus* sp. and *T. clavata* (20%) are higher than those reported by Öztürk and Altunel (2006), Aydoğdu et al. (2008), and Demirtaş and Altındağ (2011) which were 28.1%, 40%, and 83% respectively. The variation in infection levels between the two studies could be due to different host sizes and environmental factors in the areas where the fish were collected. Factors such as the size of the fish and overcrowding can significantly affect the levels of monogenean infections in their hosts, as demonstrated in studies conducted on both cultured and wild carp (Özer and Erdem, 1999).

In the current research, small-sized fish samples with an average length of 10cm were infected by different parasite species with almost high prevalence values and intensity levels. Golder et al., (1987) noticed that Small (1cm-10 cm) fish were more susceptible to *Dactylogyrus* sp. than other size groups. They also observed that the mean abundance and intensity level were highest in the smaller length group. The results of the present analysis corroborate these observations. The reason is that small and medium-sized fish have less immune power than larger fish (30.5cm-45cm).

Water temperature is commonly regarded as one of the most important factors determining the existence and abundance of monogenean parasites (Koskivaara et al., 1991), while some monogeneans tend to produce more at a higher water temperature, others prefer a lower water temperature (Hanzelova and Zitnan, 1985). *Dactylogyrus lamellatus* appears mainly among *Ctenopharyngodon idella*, which can reproduce and grow in water temperatures between 14°C to 29.5°C (Musselius and Ptasuk, 1970). Pojmanska (1995), found that the optimum period for the growth of *D. lamellatus* was in winter.

In general, species of *Dactylogyrus* show seasonal changes in their population dynamics. The seasonal variations of *Dactylogyrus* are influenced by temperature, oxygen concentrations of water, size of the fish host and fish maturity (Zitnan 1978; Pojmanska and Chabros, 1993). The present data for *D. lamellatus* (prevalence 100% and intensity 8.6 ± 3.20) recorded from grass carp in autumn (October) are significantly different from those studied by Daghigh Roohi et al (2019), who found a lower prevalence of 80.95% and a higher intensity of 14.64±5.92 from fish farms in Guilan province, north of Iran.

Additionally, our findings notably conflict with data from a similar study on a fish farm in Wuhan, China, where the prevalence was over 60% (Yao & Nie, 2004), and contradicts the result obtained from a Cyprinid aquaculture facility in N. Macedonia in autumn (Dimovska & Stojanovski, 2020), which revealed a much lower prevalence of 1.23% and a higher intensity of 26.50 compared to our research. These differences may arise from varying areas and host factors.

In North Macedonia, monogenean infestations show the highest prevalence and intensity in Lake Prespa during spring and summer (Stojanovski, 2003). This topic aligns with Thomas's (1964) findings, which suggest that the maximum prevalence and intensity of parasites occur during the vernal period due to the spawning course of cyprinid fish and the fact that female fish are physiologically more vulnerable to parasite infections during this time. However, this conclusion contrasts with the outcome yielded in the current survey.

Alterations in environmental parameters probably impact the diversity of monogenean communities between wild and farmed fish, which may influence the prevalence and mean intensity levels according to Li et al. (2018). On the other hand, the intensive culture systems are responsible for considerable stress on farmed fish due to the high stocking densities, which in turn create favorable conditions for parasite proliferation because of the increased probability of encountering the host (Ogawa, 2015).

Moreover, in artificial environments, the increase in parasitic infections has been associated with low-quality water and inadequate management (Thoney & Hargis, 1991), which coordinates with our investigation. The present study reports two more digenean parasites, *Tylodelphys clavate*, and *Diplostomum spathaceous* metacercariae, the most well-known fish digenean in Iran. They were previously found in the corpus vitreous humor in the eyes of exotic and native Cyprinids inhabiting Chaghakhour and Gandoman Lagoon (Raeisi et al., 2006).

The most significant damage caused by *Diplostomum* sp. metacercaria, is worm cataracts and subsequent growth retardation. This parasite can lead to blindness and subsequent emaciation in the fish, as they spend more time at the surface of the pond where they can be readily preyed upon by piscivorous bird species. Chinese carp in ponds are seriously threatened by eye parasites caused by *Diplostomiasis*. *Tylodelphys* sp. was first identified in the eyes of *Liza abu*, *Cyprinus carpio*, and *Barbus grypus* in Hooro-Azim, Iran, by Moghainemi (1995).

The presence of this trematode in the corpus vitreous of *Ctenopharyngodon idella* has been established by Sengupta & Dalwani (2008), Barzegar et al. (2008) and Raissy et al. (2010) into inland waters of Iran. Also, its metacercaria was previously reported from *Capoeta damascina*, *Carassius auratus*, *Aphanius isfahanensis*, and *Alburnus* sp. in Hanna Wetland, Semirom, Isfahan province (Jalali et al., 2012). Data on *Tylodelphys* spp. metacercariae from Iran is limited. This may be due to the difficulty in identifying it. However, in the current study, this parasite was identified at the species level and in a new locality according to the keys given by Gibson (1996).

Tylodelphys clavata is a digenean belonging to the family Diplostomatidae, distributed worldwide, that infects fish's eyes during its larval stage, located and feeding on the corpus vitreous, as stated by Gussev (1987). The unencysted metacercarial stage of *Tylodelphys* sp. are causing serious damage and harmful effects to the host freshwater fish, where they inhabit the eyes, the cranial cavity of the brain, the pericardial sac, the body cavity and the gas bladder (Chibwana and Nkwengulila, 2010; Chibwana et al., 2015; Otachi et al., 2015; García-Varela et al., 2016; Blasco-Costa et al., 2017).

They have a complex life cycle requiring three hosts: lymnaeid snails/ planorbid slugs as first intermediate host, a variety of fish species as second intermediate host, and the definitive hosts usually fish-eating birds and mammals (Dubois, 1970; Gibson et al., 2001; Niewiadomska and Laskowski, 2002). This parasite has been described in many fish species from the family Cyprinidae according to Barzegar (2008). The high prevalence and mean intensity of infestation occur in fish of the family Percidae. Apart from those, hosts are also the fish from the families Cobitidae, Siluridae, Salmonidae, Esocidae, Cottidae, and Clupeidae.

The following bird species, *Podiceps cristatus*, *P. caspicus*, and *P. griseigena* serve as definitive hosts for *Tylodelphys clavata* and parasitize them at an adult stage as described by Kakacheva-Avramova (1983). From an epidemiological point of view, this parasite is a Palearctic species, therefore, it may be native or transported by introducing fish (common carp or silver carp) from the Caspian Sea into other parts of the country. Tylodelphys clavata was detected in large-sized Rudd (*Scardinius erythrophthalmus*) from Lake Sapanca, Turkey (Kuş and Soylu, 2013) with a much lower prevalence of 0.62% than that observed in the present study.

This situation is probably attributed to the lake's oligo-mesotrophic conditions and Rudd's herbivorous feeding habits on macrophytes, unlike grass carp fingerlings which are zooplanktivorous (Poulin and Leung, 2011). *Tylodelphys* sp. was found to have a similar prevalence of 23% as recovered from Nile Perch (*Lates niloticus*) in Lake Nasser, southern Egypt, which is consistent with our results in autumn (Elhawary et al., 2022). This research aimed to isolate and identify parasitic infections in grass carp, as well as to determine the prevalence and intensity of these parasites. Of these parasites, one digenean metacercaria species (*Tylodelphys clavata*) found in the vitreous humor is recorded in Guilan carp culture for the first time.

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