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Grass Carp (*Ctenopharyngodon idella* Valenciennes, 1844) (Pisces: Cyprinidae) as host of new parasite species in the Ichthyoparasitofauna of Macedonia

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ABSTRACT

Grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) is one of the most important fish species from family Cyprinidae in the cyprinid aquaculture facilities in N. Macedonia. The aim of this study was to determine the presence of parasite fauna, prevalence, mean intensity, as well as, seasonal dynamic of parasite species in grass carp. A total of 76 fish specimens of grass carp were examined for parasitological investigation from the most significant and larger cyprinid aquaculture facilities in N. Macedonia. Parasite identification was performed morphologically, based on the character of significant organs, using referent keys for determination. In this study, the following parasite species were established: *Dactylogyrus lamellatus*, *Tylodelphis clavata* (larvae) and *Sinergasilus major*. The highest prevalence (35.52 %) and mean intensity (167.15) was determined with *Sinergasilus major* in autumn. The records of *Dactylogyrus lamellatus*, *Tylodelphis clavata* and *Sinergasilus major* in grass carp in the present study are considered as the first records for Macedonian aquaculture. The grass carp is regarded as a new host for these parasite species in Macedonian aquaculture. The aquaculture grass carp can potentially be considered as new host/reservoir for these parasite species in Macedonian waters. The introduction of exotic pathogens by fish imports into the country should be strictly controlled and evaluated from the outset, in order to protect not only autochthonous fish species but also the aquatic ecosystem itself.

Key words: aquaculture, parasites, fish infestation, morphological determination

Introduction

Grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) is one the most important fish species which are breeding in the cyprinid aquaculture facilities in N. Macedonia. It originates from the Far East, the Amur River and was introduced to Europe, first in the cyprinid fishponds, where it is successfully bred in polyculture with common carp, silver carp and bighead carp, and then in open waters.

According Stevanovski (2010), grass carp inhabits slow-flowing or non-running waters with lots of plankton and macro vegetation. The grass carp is an herbivore that initially feeds with zoo and phytoplankton and later exclusively on aquatic plants. Van Dyke & Sutton (1977) considered that grass carp is used as biological control agent for aquatic vegetation in aquaculture facilities.

The grass carp has not been found yet in open waters in our country. There aren't artificial hatcheries as well. The population of grass carp in cyprinid aquaculture facilities in N. Macedonia is maintained by stocking that is usually carried out by import of fish fry. Therefore, special care

should be taken in future when buying stocking fish material from other countries.

In fish farms in Bosnia and Hercegovina, according to literature reviews, the presence of parasite species in *Ctenopharyngodon idella* has been established by several authors. Žitnan et al. (1969) determined the presence of *Ichthyophthirius multifiliis*, *Dactylogyrus lamellatus* and *Argulus foliaceus* in *Ctenopharyngodon idella* from fish farms in Bosnia and Hercegovina; Kiškaroly and Tafro (1983, 1988) established the presence of *Diplostomum spathaceum* and *Balantidium ctenopharyngodonis*; Kiškaroly et al. (1980) concluded that *Dactylogyrus lamellatus* is most common in grass carp from fish farms, with prevalence of 13.03 %; Kiškaroly et al. (1987) found the presence of *Dactylogyrus lamellatus* and *D. ctenopharyngodonis*.

Hristovski (1983) examined the helminthes fauna in *Ctenopharyngodon idella* from cyprinid fish farms in Macedonia and determined the presence of *Bothriocephalus opsariichthydis* and *Contracaecum* sp.

According to the literature reviews in the world, Ali et al. (1988) found *Apiosoma piscicola*, *Chilodenella cyprini*, *Ichthyophthirius multifiliis*, *Trichodina domerguei* and *Lernaea cyprinacea* in grass carp from fish ponds in Iraq. Tasawar et al. (2009) examined the prevalence of ectoparasite infestation of the genus *Lernaea* at *Ctenopharyngodon idella* in a fish pond in Multan, Pakistan, and found four parasite species: *Lernaea cyprinacea* (6,53%), *L. polymorpha* (7,54%), *L. oryzophila* (0,67%) and *L. lophiara* (0,67%). Abd El-Galil (2002) found the presence of copepod *Lernaea cyprinacea* in the offspring of *Ctenopharyngodon idella* in fishponds in Egypt, with a prevalence of 32 %. Bozorgnia et al. (2012) performed parasitological examinations in a fishpond in Iran and found the presence of *Trichodina perforate* and *Dactylogyrus lamellatus* in *Ctenopharyngodon idella*. Shamsi et al. (2009) determined the presence of *Dactylogyrus ctenopharyngodonis* and *D. lamellatus* in *Ctenopharyngodon idella* of cyprinid fish pond in several regions of Iran. Sengupta and Dalwani (2008) examined the parasite fauna in *Ctenopharyngodon idella* from the lagoon Choghakhor, Iran and determined the presence of *Dactylogyrus lamellatus* (on gills), metacercariae of *Diplostomum spathaceum* (eye lens) and metacercariae of *Tylodelphys clavata* (corpus vitreum). Barzegar et al. (2008) found metacercariae of *Tylodelphys clavata* in corpus vitreum of *Ctenopharyngodon idella* in Iran. Xi et al. (2011) determined the presence of the cestod *Bothriocephalus opsarichthydis* in *Ctenopharyngodon idella* from the river Changjiang, China, with mean intensity of 36.9 ± 54.7 . Nie and Yao (2000) examined the seasonal dynamic of copepods from genus *Sinergasilus* in *Ctenopharyngodon idella* from fish pond in China and found the presence of *Sinergasilus major* during the summer. Yao & Nie (2004) determined the presence of *Dactylogyrus lamellatus* on gills in *Ctenopharyngodon idella* from a fishpond at the Institute of Hydrobiology in Wuhan, China. They noticed that the prevalence with this parasite was higher than 60%, while the mean intensity increased gradually from November to March, with the highest peak in April. Li et al. (2008) determined the presence of *Apiosoma piscicola* in *Ctenopharyngodon idella* from fish pond in China. Lazar (2009) identified the presence of *Lernaea* sp. on skin of *Ctenopharyngodon idella* from fish ponds in Romania.

Materials and Methods

This study was carried out by four seasons on adult fish. The fish were caught using net. The specimens were placed in plastic containers and transferred alive to the laboratory.

The aim of this study was to determine the presence of parasite fauna, prevalence, mean intensity, as well as, seasonal dynamic of parasite species in grass carp (*Ctenopharyngodon idella*) from the most significant and

larger cyprinid aquaculture facilities in N. Macedonia.



Figure 1. Grass carp (*Ctenopharyngodon idella*) (original).



Figure 2. Sampling points – cyprinid aquaculture facilities.

Only fresh fishes were subjected of routine identification, dissection and observation methods. Cleaned parasites were separated and put in certain fixatives, prepared for the determination with determined techniques of staining and clearing (Vasiljkov, 1983; Gussev, 1983).

For identification of the 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 and mean intensity) were calculated according to Bush et al. (1997).

During the examinations at Department of Fish Pathology from Hydrobiological Institute in Ohrid (N. Macedonia), stereomicroscopes „Zeiss”- Stemi DV4 and „MBS 10”, as well as light microscope „Reichert” were used.

Results

A total of 76 fish specimens of grass carp were examined for parasitological investigation from the most significant and larger cyprinid aquaculture facilities in N. Macedonia (Figure 2). In this study, the following parasite species were established: *Dactylogyrus lamellatus*, *Tylodelphis clavata* (larvae) and *Sinergasilus major* (Table 1 and 2).

In our study, *Dactylogyrus lamellatus* was found on gills in 23 specimens of grass carp in spring and autumn.

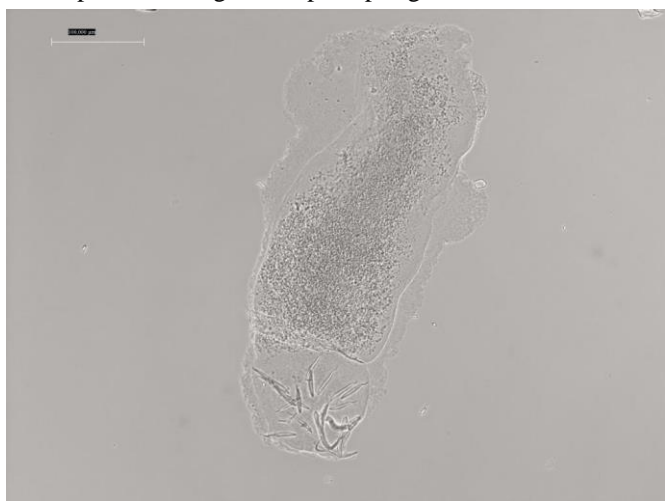


Figure 3. *Dactylogyrus lamellatus* on gills in *Ctenopharyngodon idella* – whole parasite (original).



Figure 4. *Dactylogyrus lamellatus* on gills in *Ctenopharyngodon idella* – whole parasite (original).



Figure 5. *Dactylogyrus lamellatus* on gills in *Ctenopharyngodon idella* – copulatory organ (original).

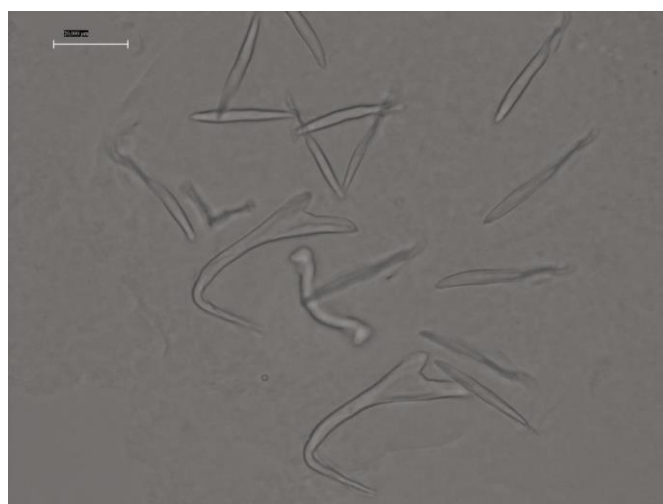


Figure 6. *Dactylogyrus lamellatus* on gills in *Ctenopharyngodon idella* – hooks (original).

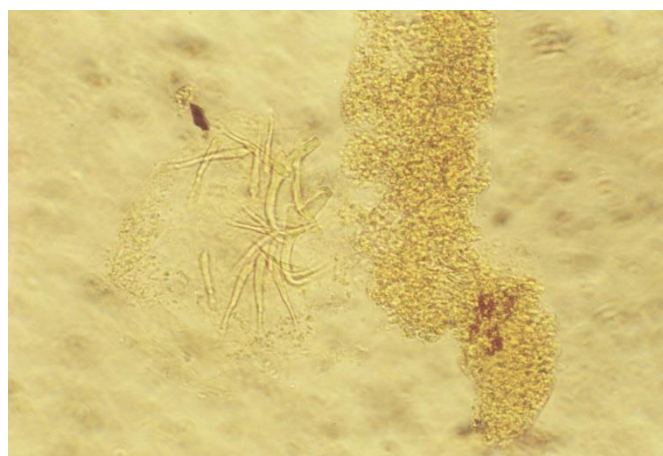


Figure 7. *Dactylogyrus lamellatus* on gills in *Ctenopharyngodon idella* – hooks (original).

In our study, *Tylodelphis clavata* (larvae) was found in the corpus vitreous of 18 specimens of *Ctenopharyngodon idella*, only in autumn.



Figure 8. *Tylodelphis clavata* in eye of *Ctenopharyngodon idella* – whole parasite (original).

In our study, *Sinergasilus major* was found on fins and gills in 27 specimens of grass carp in autumn.



Figure 9. *Sinergasilus major* in *Ctenopharyngodon idella* – whole parasite (original).



Figure 10. *Sinergasilus major* in *Ctenopharyngodon idella* – hooks (original).



Figure 11. *Sinergasilus major* in *Ctenopharyngodon idella* – hooks (original).



Figure 12. *Sinergasilus major* in *Ctenopharyngodon idella* (original).

Table 1. Prevalence and mean intensity with parasite species in grass carp (*Ctenopharyngodon idella*) from cyprinid aquaculture facilities in N. Macedonia.

Parasite species	Number of examined fish	Number of infected fish	Mean intensity	Prevalence (%)
1 <i>Dactylogyrus lamellatus</i>		23	19.00	30.26
2 <i>Sinergasilus major</i>	76	27	167.15	35.52
3 <i>Tylodelphys clavata</i>		18	4.78	23.68

Table 2. Prevalence (E) and mean intensity (I) with parasite species in grass carp (*Ctenopharyngodon idella*) from cyprinid aquaculture facilities in N. Macedonia, by seasons.

Parasite species	Spring		Summer		Autumn		Winter	
	I	E (%)	I	E (%)	I	E (%)	I	E (%)
<i>Dactylogyrus lamellatus</i>	7.33	0.79	/	/	26.50	1.23	/	/
<i>Sinergasilus major</i>	/	/	/	/	167,15	35.53	/	/
<i>Tylodelphys clavata</i>	/	/	/	/	4.78	1.59	/	/

Discussion

Dactylogyrus represents the most dominant genus among the class Monogenea. This genus includes more than 200 parasite species that are characterized by different pathogenicity. Dove and Ernst (1998) considered that *Dactylogyrus* is one of the largest genera of parasitic helminths, of which 95% are parasites of gills in the fish of family Cyprinidae.

The highest prevalence (35.52 %) and mean intensity (167.15) in grass carp (*Ctenopharyngodon idella*) was determined with *Sinergasilus major* in autumn. The lowest prevalence (23.68 %) and mean intensity (4.78) was established with *Tylodelphys clavata*, also in autumn (Table 1 and 2).

Dactylogyrus lamellatus was found in *Ctenopharyngodon idella* by Sengupta and Dalwani (2008), Shamsi et al. (2009) and Bozorgnia et al. (2012) in fish farms in Iran and by Yao and Nie (2004) in a fish farm in China, with prevalence higher than 60% and the highest culmination of the mean intensity in April.

Tylodelphys clavata is a digenea that belongs to the family Diplostomatidae and parasites in the fish eyes, where

during the larval stage it is located and fed in the corpus vitreous of the eye (Gussev, 1987). According Barzegar (2008), this parasite has been identified in a large number of fish species from the family Cyprinidae, as well as in many aquaculture birds. Shahryari (2002) noticed that *Tylodelphys clavata* in the stadium of metacercariae, parasitizing the fish as a second host, causing eyes damages.

According Kakacheva-Avramova (1983), *Tylodelphys clavata* parasitizes as adult stage in following birds' species: *Podiceps cristatus*, *P. caspicus* and *P. griseigena*. The development is quite complex. The first intermediate host is *Radix ovata*, and the second is fish. The highest prevalence and mean intensity of infestation occurs in fish of family Percidae. Apart those, hosts are also the fish from the families Cyprinidae, Cobitidae, Siluridae, Salmonidae, Esocidae, Cottidae and Clupeidae.

Most eye parasites can cause severe blindness in fish. Although infected fish do not die directly from these parasites, the effects of infection (slow growth, behavioral changes, and secondary invasions by fish-eating birds, bacteria, and protozoa) can cause high mortality. In the fish farms where *Cyprinus carpio*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* are reared, there is a serious danger of parasites attacking the eyes, which is a causative agent of blindness and atrophy. As a result of swimming near

the water surface, they can easily become prey to fish-eating birds.

The presence of metacercariae of this parasite in the corpus vitreous of *Ctenopharyngodon idella* has been established by Sengupta & Dalwani (2008), Barzegar et al. (2008) and Raissy et al. (2010) in waters in Iran.

According to Chang et al. (2005), the parasitic copepod *Sinergasilus major* is a very important pathogen for the grass carp. *Sinergasilus major* is commonly found on the gills of grass carp, less often in other fish species from the Amur River and the rivers of the Far East. With the spreading worldwide of the host, the parasite has been introduced in many parts of the world and represents a threat to the host health. At high mean intensity (over 200 parasites in two-year-old fish), *Sinergasilus major* can cause disease and high fish mortality.

Data on the presence of *Sinergasilus major* in *Ctenopharyngodon idella* were published by Bauer & Babaev (1964) in waters in Central Asia and Russia, as well as by Nie & Yao (2000) in fish farms in China.

Grass carp is mainly cultured in N. Macedonia for aquatic weed control, rather than for human consumption.

The records of *Dactylogyrus lamellatus*, *Tylodelphis clavata* and *Sinergasilus major* in grass carp (*Ctenopharyngodon idella*) in the present study are considered as the first records in N. Macedonia. The grass carp is regarded as a new host for these parasite species in Macedonian aquaculture.

The prevalence with *Dactylogyrus lamellatus* in grass carp was 30.26 %, while the mean intensity 19.00. The prevalence with *Tylodelphis clavata* (larvae) in grass carp was 23.68 %, while the mean intensity 4.78. The prevalence with *Sinergasilus major* in grass carp was 35.53%, while the mean intensity 167.15.

Due to the fact that most of the parasites found in our work are associated to their host, which is native to eastern Asia, we regard that uncontrolled imports of live fish into the country might contribute to the transmission of various parasites to autochthonous fish species, causing great economic and environmental damage to the fish population. Therefore, special care should be taken when buying stocking fish material from other countries.

The introduction of exotic pathogens by fish imports into the country should be strictly controlled and evaluated from the outset, in order to protect not only autochthonous fish species but also the aquatic ecosystem itself.

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