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DISTRIBUTION OF *EUDIPLOZOON NIPPONICUM* (MONOGENEA, DIPLOZOIDAE) IN FARMED COMMON CARP (*CYPRINUS CARPIO*, L. 1758) FROM AQUACULTURE FACILITIES IN MACEDONIA

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Original scientific paper

Abstract: A total of 958 specimens of common carp from the most significant and larger cyprinid aquaculture facilities in Macedonia, including fish farms and reservoirs, were examined for parasitological investigations. *Eudiplozoon nipponicum* was found on gills in 121 specimens of common carp in spring, summer and autumn, with a prevalence of 10.67 %, and a mean intensity of 2.55. Our findings of *E. nipponicum* in common carp from aquaculture are first recorded in Macedonia.

Key words: diplozoon, parasites, common carp, aquaculture

Introduction

Diplozoids (Diplozoidae, Monogenea) are fish ectoparasites with a direct life cycle without intermediate hosts. Their free-swimming larva is called oncomiracidium. It hatches from eggs, invades a fish host and metamorphoses into a post-oncomiracidial larval stage, which is called dipopra. During their adult life, two dipoprae fuse and live as a pair in cross-copulation (*Pečínková et al., 2007*).

A typical and unique representative of the genus *Eudiplozoon* which belongs to the family Diplozoidae is *Eudiplozoon nipponicum*. According to *Matejusova et al. (2004)*, about 18 parasite species belonging to 2 genera, *Diplozoon* and *Paradiplozoon*, have been identified in Europe. *Denis et al. (1983)* considered that *Eudiplozoon nipponicum* has been introduced through the importation of carp from Asia to Europe.

Valigurová et al. (2011) state that the parasite has a complex digestive tract that is well equipped for hematophageal nutrition. According to *Milne and Avenant-Oldewage (2012)*, *Eudiplozoon nipponicum* infestation occurs as soon as the potential host swims through a group of oncomiracidiums. After attachment on

the host gills, the oncomiracidium develops into a dipopra with two pairs of clamps and can only reach maturity after merging with another dipopra, and the complete set of four pairs of clamps develops during the maturation phase.

Hermaphroditic adult forms are constantly fused, forming a characteristic "X" shape. They divide the reproductive and digestive systems (*Kamegai, 1976*). Such a reproductive strategy, in which two independent heterogeneous individuals unite in a hermaphroditic organism, without the need to look for a mating partner, demonstrates the high specialization of diploids to their parasitic life. Haptoral attachment flaps are permanent structures that are present at all stages of the diploid life cycle, from free-floating to parasitic adult stages (*Khotenovsky, 1977*).

The hatched larva of the oncomiracidium settles on the gills of the host fish, followed by transformation into a dipopra larva. The ventral growth is formed after three days and the mating of the two dipopra occurs after four days at T of 25°C (*Hirose et al., 1987*). The parasite attaches with the help of four pairs of clamps to the posterior part of the body and feeds on the blood of the host.

This study aimed to determine the distribution of *Eudiplozoon nipponicum*, prevalence, mean intensity, as well as, seasonal dynamic in farmed common carp (*Cyprinus carpio*, L. 1758) from the aquaculture facilities in Macedonia.

Material and Methods

This parasitological study was carried out by seasons, in three years. A total of 958 specimens of common carp from the most significant and larger cyprinid aquaculture facilities in Macedonia, including fish farms and reservoirs, were examined for parasitological investigations (Fig. 1). Only fresh fishes were subjected to routine identification, dissection and observation methods. Cleaned parasites were separated and put in certain fixatives, prepared for determination with determined techniques of staining and clearing (*Vasiljkov, 1983; Gussev, 1983; Stojanovski, 1997, 2003*). The gill filaments of fish were inspected by stereomicroscope. All parasites found in each fish were identified and enumerated. Preparation, fixing, staining and mounting of parasites for morphological determination was made by common methods used in parasitology. Classical epidemiological variables (prevalence and mean intensity) were calculated according to *Bush et al. (1997)*. The parasite specimens were identified according to reference keys of *Bauer (1985)* and *Gussev (1983)*.

During the examinations at Laboratory for fish diseases in Hydrobiological Institute in Ohrid (Macedonia), stereomicroscopes „Zeiss”- Stemi DV4 and „MBS 10”, as well as, light microscope „Reichart” were used.

Data of the number of fish examined, fish infected, prevalence and mean intensity (total and by seasons) are given in Tables 1, 2 and 3.

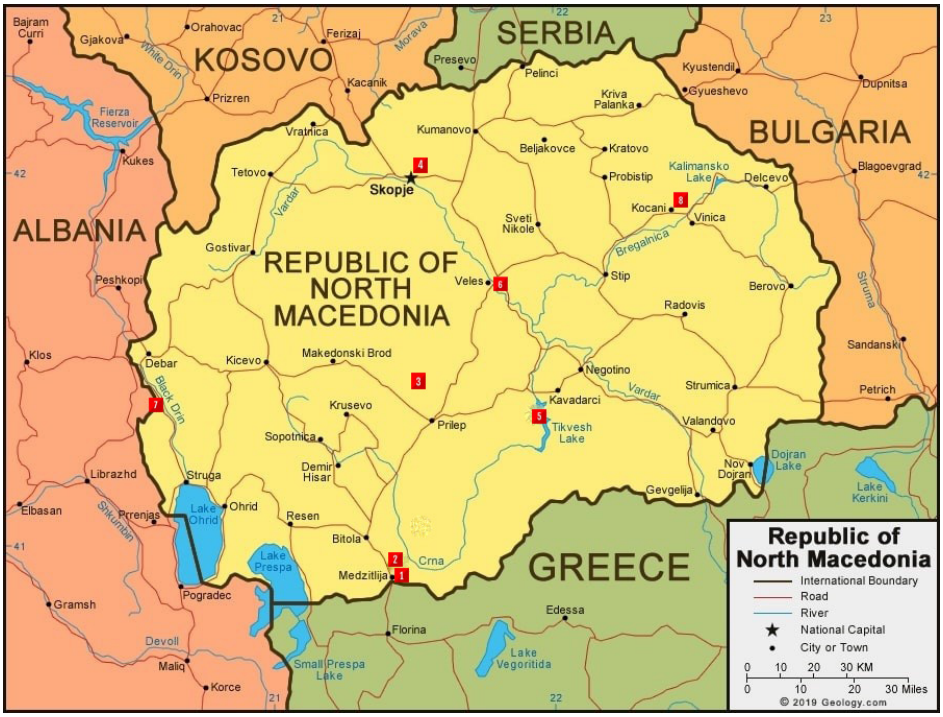

Fig.1. Sampling points –aquaculture facilities
(Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)

Legend:
1 – Fish pond Zhabeni
2 – Fish pond Bukri
3 – Fish pond Dolneni
4 – Fish pond Zhelezara
5 – Tikvesh reservoir
6 – Mladost reservoir
7 – Globochica reservoir
8 – Gradche reservoir

Results and Discussion

A total of 958 fish specimens of common carp from the most significant and larger cyprinid aquaculture facilities in Macedonia were comprised by a parasitological investigation (Fig. 1). In this study, *Eudiplozoon nipponicum* was found on gills in 121 specimens of common carp in spring, summer and autumn.

Our findings of *Eudiplozoon nipponicum* in common carp from aquaculture are first recorded in Macedonia.

Table 1. Determination of *Eudiplozoon nipponicum* by aquaculture facility and season

Season / Aquaculture facility	Spring	Summer	Autumn
Zhabeni	√	√	
Bukri		√	√
Dolneni			√
Zhelezara			√
Tikvesh Reservoir	√		
Mladost Reservoir	√		√
Globochica Reservoir	√		
Gradche Reservoir			√

Table 2. Total prevalence and mean intensity with *Eudiplozoon nipponicum* in common carp(*Cyprinus carpio,* L. 1758) from aquaculture facility

Fish species	Number of examined fish	Number of infected fish	Mean intensity	Prevalence (%)
Common carp (*Cyprinus carpio*, L. 1758)	958	121	2.55	10.67

Table 3. Prevalence (P) and mean intensity (I) with *Eudiplozoon nipponicum* in common carp (*Cyprinus carpio*, L. 1758) from aquaculture facility, by season

Parasite species	Spring		Summer		Autumn		Winter	
	I	P (%)	I	P (%)	I	P (%)	I	P (%)
Eudiplozoon nipponicum	1.89	4.76	2.39	6.53	3.70	11.73	/	/

The prevalence with *Eudiplozoon nipponicum* in common carp was 10.67 %, while the mean intensity was 2.55.

The prevalence with *Eudiplozoon nipponicum* in common carp by seasons was as following: spring – 4.76 %, summer – 6.53 %, autumn – 11.73 %; while the mean intensity was: spring – 1.89, summer – 2.39 and autumn – 3.70.

Eudiplozoon nipponicum has the following dimensions: body length 4.4 - 5.4 mm; length of the anterior part 3.0 - 3.5 mm; length of posterior part 2.0 - 2.2 mm. There are two suckers on the anterior part of the body with a diameter of 0.09 - 0.11 mm. Characteristic of *Eudiplozoon nipponicum* are the two circular glandular formations in front of the suckers. The posterior part of the body consists of three parts. One part of the body has 12 - 14 large wrinkles, which are equal to each other on both sides. The middle part forms an enlargement, on which there are large lateral oblique wrinkles. The other part is provided with four adhesive clamps

with the following dimensions: I - 0.08 × 0.12 mm; II - 0.07 × 0.15 mm; III - 0.09 × 0.14 mm; IV - 0.08 × 0.14 mm. The middle hooks are 0.024 mm long.

The covering of free-floating oncomiracidiums occurs in two types: ciliary and non-ciliary, with numerous non-ciliary sensory structures. The attachment apparatus begins to form during the oncomyracidium stage. The cover groove becomes visible later and plays a role in attaching the parasites. According to the reproductive strategy of *Eudiplozoon nipponicum*, a very important role is played by the two morphological structures of the dipopra (ventral suction cup and dorsal papilla). The posterior part of the body in adult parasites is highly modified for attachment. Haptor, grooves, and lobular dilatations are highly developed. The anterior part of the body is flexible and able to connect with the host gill tissue through the mouth and associated oral structures.

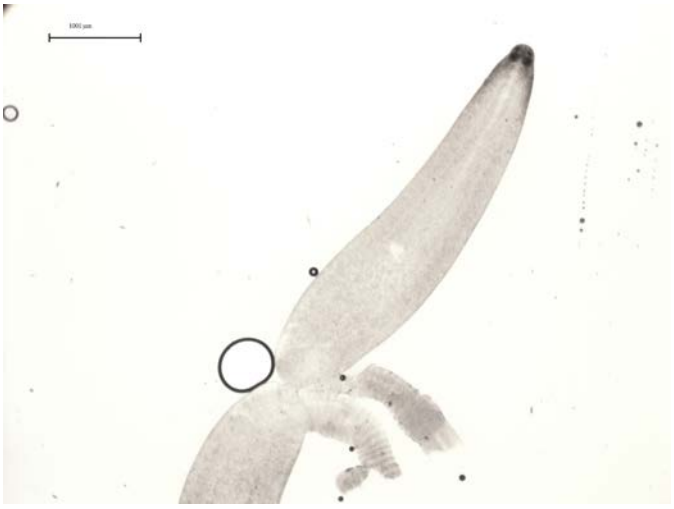

Fig. 2. *Eudiplozoon nipponicum* in common carp (whole parasite) (Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)

Fig. 3. ***Eudiplozoon nipponicum*** **in common carp (whole parasite) (Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)**

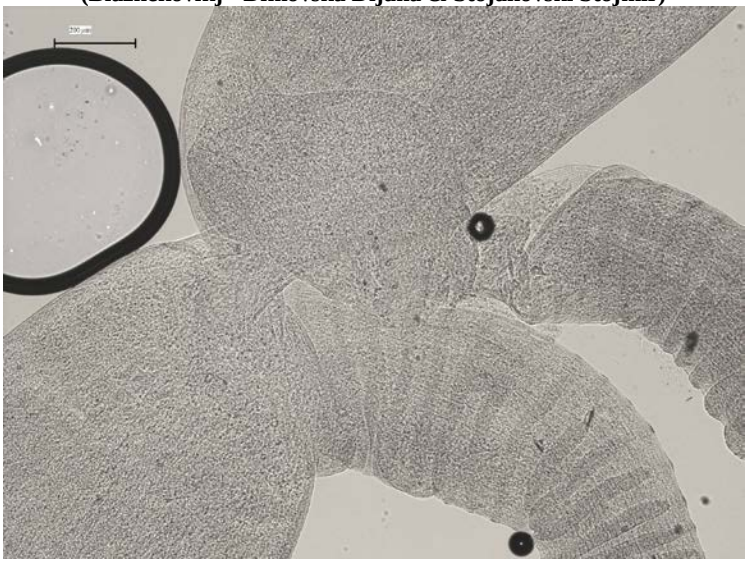

Fig. 4. ***Eudiplozoon nipponicum*** **in common carp (whole parasite) (Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)**

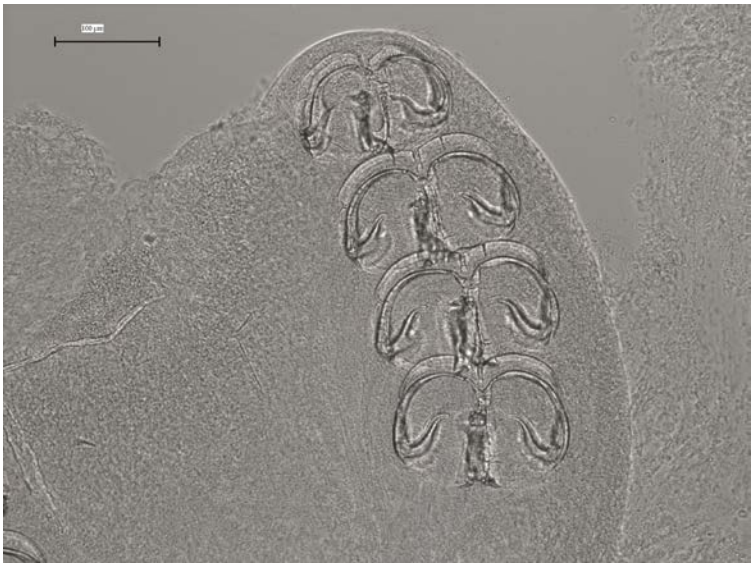

Fig. 5. *Eudiplozoon nipponicum* in common carp (4 clamps) (Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)

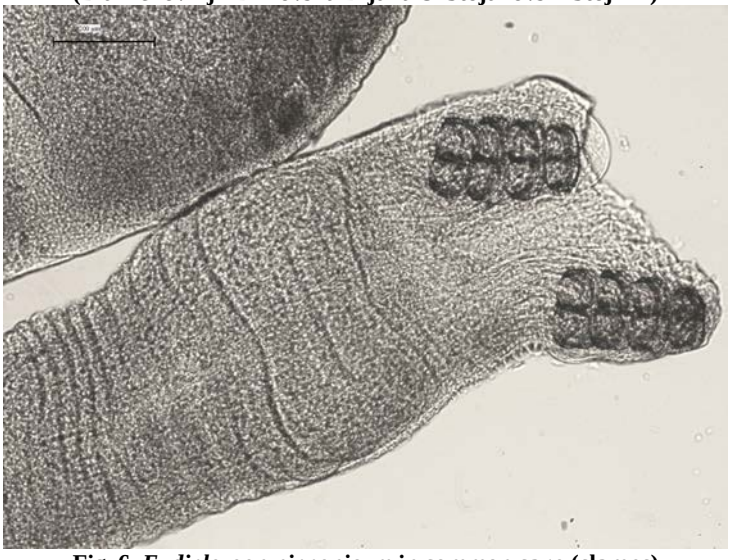

Fig. 6. *Eudiplozoon nipponicum* in common carp (clamps) (Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)

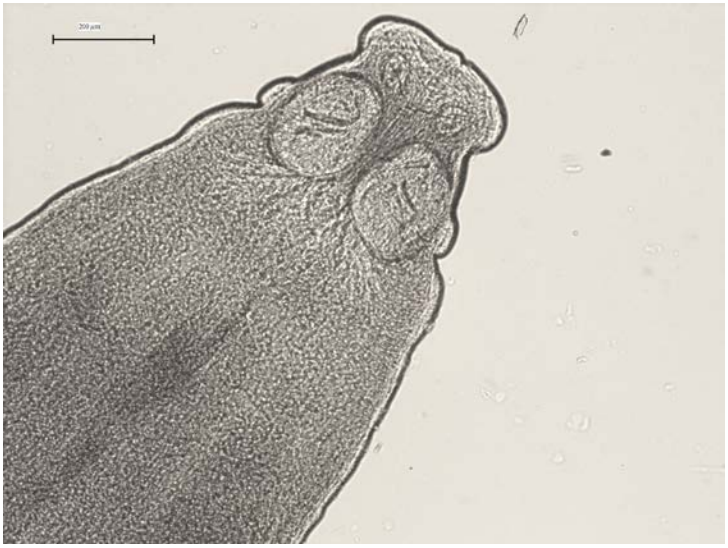

Fig. 7. ***Eudiplozoon nipponicum*** **in common carp (glandular formations on the anterior part) (Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)**

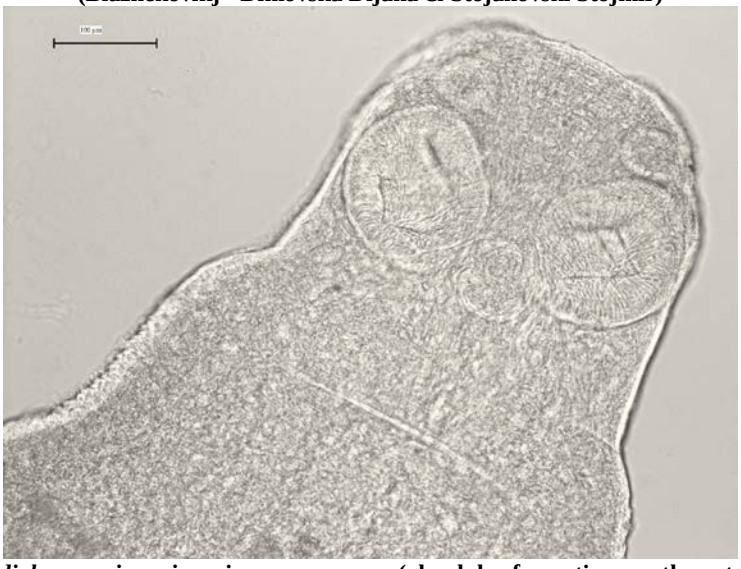

Fig. 8. ***Eudiplozoon nipponicum*** **in common carp (glandular formations on the anterior part) (Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)**

According to the data from previous parasitological researches in Macedonia, *Eudiplozoon nipponicum* in common carp was first determined by *Stojanovski (2003)* in Lake Prespa, with a prevalence of 3.58% and mean intensity of 3.25, as well as, in Lake Dojran, with a prevalence of 1.76% and mean intensity of 1.31. Also, tha data for the presence of *Eudiplozoon nipponicum* established

Hristovski et al. (2003) during the parasitological examinations in Lake Dojran Lake and *Hristovski et al. (2006, 2012)* during parasitological examinations in Lake Prespa, with a prevalence of 44.4%.

According to world literary reviews, the presence of *Eudiplozon niponicum* in common carp has been established by *Nedeva (1991)* in waters in Bulgaria; *Szekely and Molnar (1996 - 1997)* in Lake Balaton, Hungary and *Rohlenová et al. (2011)* and *Ondračková et al. (2012)* in fishponds in the Czech Republic.

According to *Kawatsu (1978)*, infested fish develop hypochromic microcytic anemia characterized by an increase in immature erythrocytes, which can lead to death, especially in carp juveniles. *Shindo (1997)* states that there is hyperplasia around the place where the parasite is attached to the gill epithelium, as we noticed in our research.

Conclusion

A total of 958 specimens of common carp from the most significant and larger cyprinid aquaculture facilities in Macedonia, including fish farms and reservoirs, were examined for parasitological investigations. *Eudiplozoon nipponicum* was found on gills in 121 specimens of common carp in spring, summer and autumn, with a prevalence of 10.67 %, and a mean intensity of 2.55.

In our study, hyperplasia of the gill epithelium and pale gill around the place where the parasite is attached to the gill epithelium was noticed, but not more serious damage to fish health or higher percentage of mortality.

Our findings of *Eudiplozoon nipponicum* in common carp from aquaculture are first recorded in Macedonia.

Due to the high number of parasites found and the pathological changes they cause, continuous follow-up of the condition with the presence of *Eudiplozoon nipponicum* in fish from aquaculture facilities is required.

Rasprostranjenost *Eudiplozoon nipponicum* (monogenea, diplozoidae) u gajenom običnom šaranu (*Cyprinus carpio*, l. 1758) iz objekata za akvakulturu u Makedoniji

Dijana Blažekovikj - Dimovska, Stojmir Stojanovski

Rezime

U okviru parazitološkog istraživanja, ispitano je ukupno 958 primeraka običnog šarana iz najznačajnijih i većih objekata akvakulture u Makedoniji, uključujući

ribnjake i rezervoare. *Eudiplozoon nipponicum* pronađen je na škrgama u 121 primerku običnog šarana u proleće, leto i jesen, sa prevalencijom od 10,67%, i srednjim intenzitetom od 2,55. Naši nalazi *E. nipponicum* kod običnog šarana iz akvakulture prvi put su zabeleženi u Makedoniji.

Ključne reči: diplozoon, paraziti, šaran, akvakultura

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