

# Parasite Fauna of Chub *Squalius squalus* (Bonaparte, 1837) (Actinopterygii: Leuciscidae) from Belčišta Wetland, Republic of North Macedonia

Stojmir Stojanovski<sup>1,\*</sup>, Lidija Velkova-Jordanoska<sup>1</sup> & Dijana Blazhekovic-Dimovska<sup>2</sup>

<sup>1</sup> Hydrobiological Institute, Ohrid, Republic of North Macedonia; E-mail: [stojstoi@gmail.com](mailto:stojstoi@gmail.com); [lidvejo@yahoo.com](mailto:lidvejo@yahoo.com)

<sup>2</sup> Faculty of Biotechnical Sciences, University St. Kliment Ohridski, Bitola, Republic of North Macedonia;  
E-mail: [dijanablazekovic@yahoo.com](mailto:dijanablazekovic@yahoo.com)

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**Abstract:** During a parasitological survey of *Squalius squalus* from Belčišta Wetland (Republic of North Macedonia), six parasite species were found. These were *Dactylogyrus sphyrna*, *Paradiplozoon ergensi*, *Gyrodactylus* sp., *Allocreadium isoporum*, *Proteocephalus torulosus* and *Pomphorhynchus bosniacus*. The total helminth prevalence was 90.91%. The species with the highest prevalence were *P. bosniacus* (59.09%) and *D. sphyrna* (54.55%). The average intensity of infection was 6.08, with the highest values of *D. sphyrna* (7.83) and *P. bosniacus* (5.92). The present study is the first record of *Proteocephalus torulosus* in *S. squalus* in the Republic of North Macedonia. Among the parasite species found, the greatest pathological impact was associated with *D. sphyrna*, *P. ergensi* and *P. bosniacus*.

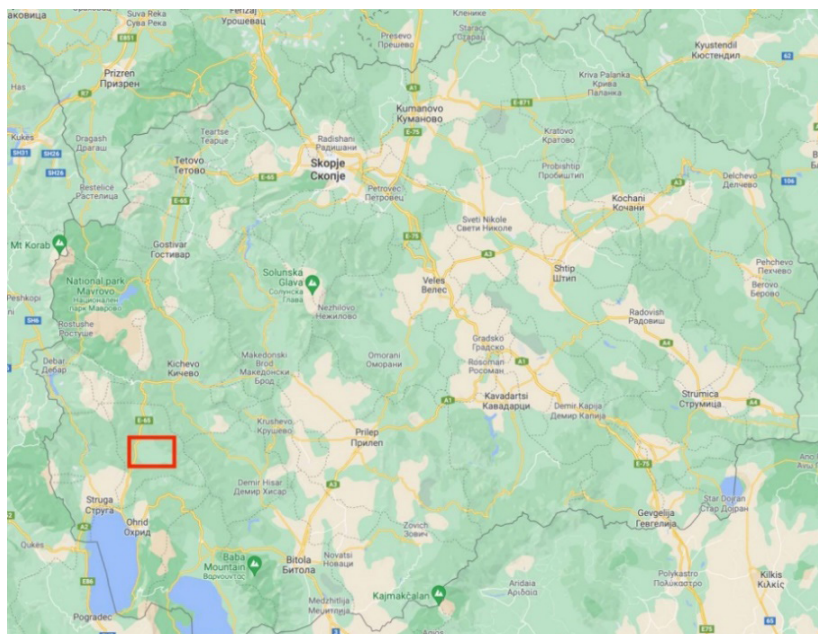
**Key words:** parasite fauna, chub, Belčišta Wetland

## Introduction

Belčišta Wetland is located at an altitude of 767 m in the municipality of Debrca at the foot of Ilinska Mountain (Fig. 1). The wetland is fed by 11 karstic springs (known as Sini Viroj), which originate from the springs north and northeast of the village of Novo Selo, in the direction of the village of Belčišta. The wetland covers around 400 hectares. There are also several lakes in the wetland (Fig. 2), some of them being larger: Belčiški Sin Vir, Novoselski Sin Vir 1, Novoselski Sin Vir 2 and Sino Duvlo. Belčišta Wetland is a remnant of the former Desaret Lake, which

flooded the Debrca Valley in the Pliocene. It is a part of the lake region of South Macedonia and Thessaly, which includes the lakes Ohrid, Prespa and Malič and belongs to the Adriatic Sea basin. This wetland is the largest and one of the most important in North Macedonia, with flooded forests and wet pastures, mostly known for its alder forests. With the retreat of the Desaret Lake along the Sateska River, many endemic plant and animal species remained isolated in this wetland. In this water ecosystem, a total of 55 plant species and seven fish species have been recorded. There are also 9 species of birds, 9 species of mammals, 9 amphibian and reptile species and 14

\*Corresponding author: [stojstoi@gmail.com](mailto:stojstoi@gmail.com)



**Fig. 1.** Map of the Republic of North Macedonia, with indication of the position of the Belčišta Wetland (rectangle). Source: Google Maps.

invertebrate species of high conservational importance. Belčišta Wetland is included in the Emerald National Network of the Republic of North Macedonia and is proposed to be included into Natura 2000 network (Zoroski 2002).

Earlier investigations of the parasite fauna in fishes in the Belčišta Wetland were carried out by Blazhekovikj-Dimovska et al. (2022). They recorded *Pomphorhynchus bosniacus* Kiškarolj & Čanković, 1969 from *Squalius squalus* (Bonaparte, 1837), *Phoxinus lumaireul* (Schinz, 1840) and *Pelagus minutus* (S. L. Karaman, 1924). Later, Blazhekovikj-Dimovska et al. (2023) found *Acanthocephalus lucii* (Müller, 1776) in *Phoxinus lumaireul* from the same area.

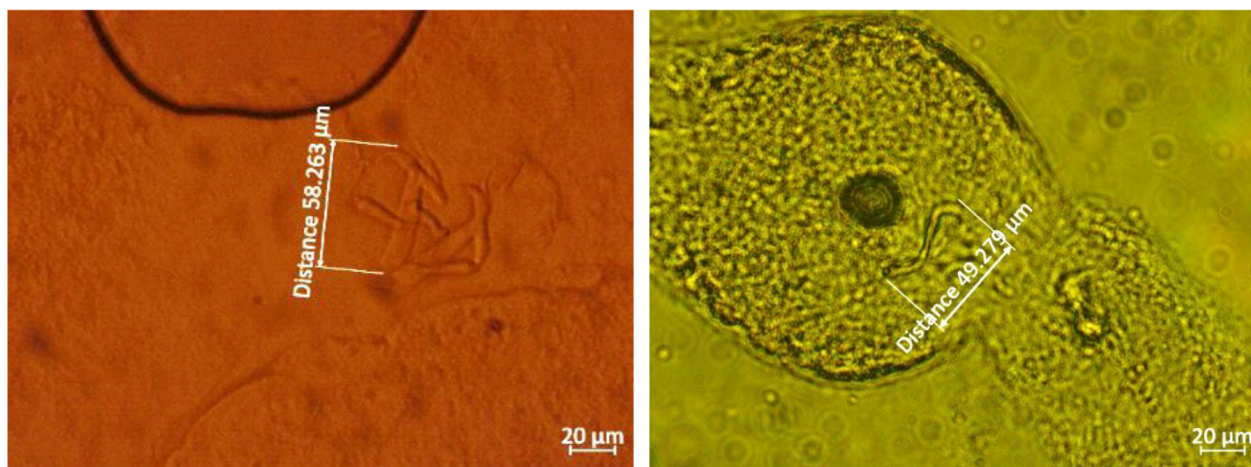
From adjacent water bodies, Hristovski (1983) reported four helminth species from *Squalius prespensis* (Fowler, 1977) (= *Leuciscus cephalus prespensis* Fowler, 1977) from Lake Prespa: the cestode *Ligula intestinalis* (L., 1758) (larva), the nematodes *Cystidicoloides tenuissima* (Zeder, 1800) and *Contracaecum microcephalum* (Rudolphi, 1809) (larva), and the acanthocephalan *Pomphorhynchus bosniacus*. He also found the cestode *L. intestinalis* (larva), the nematode *C. microcephalum* (larva) and the acanthocephalans *Metechinorhynchus truttae* (Schränk, 1788), *Acanthocephalus anguillae* (Müller, 1780) and *P. bosniacus* in *S. squalus* from the nearby Lake Ohrid. Dupont & Lambert (1986) recorded two monogeneans, *Dactylogyrus prostae* and *D. vistulae*, in *S. prespensis* from Lake Prespa. Stojanovski et al. (2012) reported 13 parasite



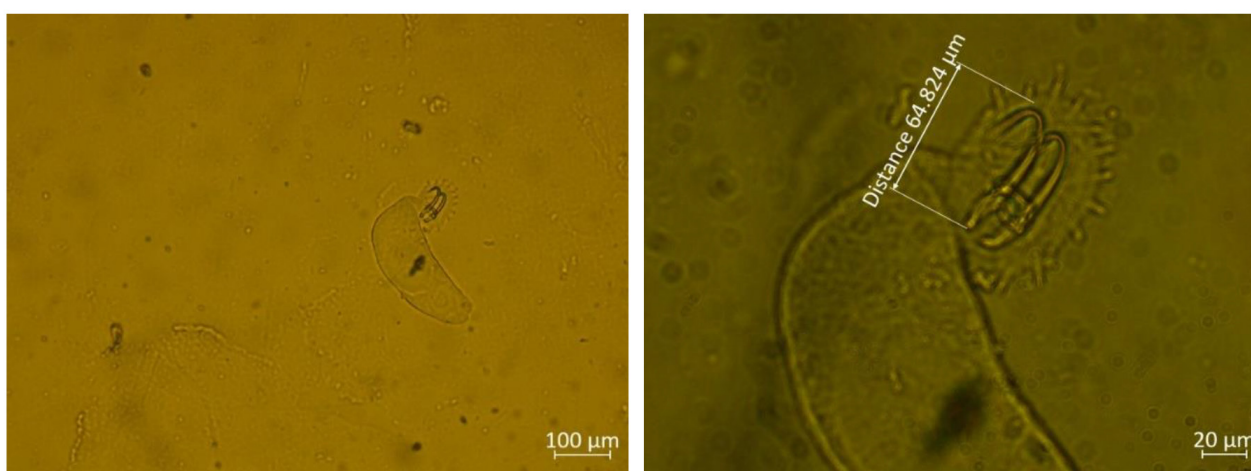
**Fig. 2.** Belčišta Wetland, typical wetland landscapes

species from *Squalius squalus* from Lake Ohrid: *Myxobolus muelleri*, *Dactylogyrus sphyryna*, *D. folkmanovae*, *D. vistulae*, *Paradiplozoon ergensi*, *Allocreadium isoporum*, *Ligula intestinalis* (plerocercoid), *Philometra ovata*, *Raphidascaris acus*,





**Fig. 3.** *Dactylogyrus sphyrna* von Linstow, 1878: hard parts of the haptor (left) and copulatory organ (right).



**Fig. 4.** *Gyrodactylus* sp.: general view (left) and haptor (right).

*Contracaecum microcephalum* (larva), *Metechinorhynchus truttae*, *Acanthocephalus anguillae* and *Pomphorhynchus bosniacus*. Stojanovski (2003) reported the monogeneans *D. sphyrna* and *D. prostaе* as parasites of *S. prespensis* from Lake Prespa.

In view of the more species-rich parasite fauna recorded in the adjacent waters, it is obvious that the parasite fauna in fishes in the Belčišta Wetland is incompletely examined. The aim of the present study is to examine the diversity of the parasite species occurring in *Squalius squalus* from Belčišta Wetland.

## Materials and Methods

Totally, 44 specimens of chub *Squalius squalus* from Belčišta Wetland were examined for parasites in different seasons in 2021–2023. The studied localities were Djošev Kladenec, Novoselski Vir and Matica (near the bridge where it flows into the Sateska River). Only fresh fish were subjected to routine identi-

fication and dissection methods. The parasites were isolated using stereomicroscope Zeiss Stemi 305 and microscope Zeiss Primovert. They were fixed in 80 % ethanol. The flatworm parasites were stained, cleared and mounted according to the methods described by Vasiljkov (1983) and Gushev (1983). For morphological examination, permanent slides of whole individual parasites were prepared by staining with acetocarmine, dehydrating with ascending grades of alcohol and mounting in Canada balsam. Identification of acanthocephalans was based on the morphology of neck, bulb, proboscis, hooks and reproductive system. We used identification keys by Bauer (1985, 1987) and Čanković et al. (1968).

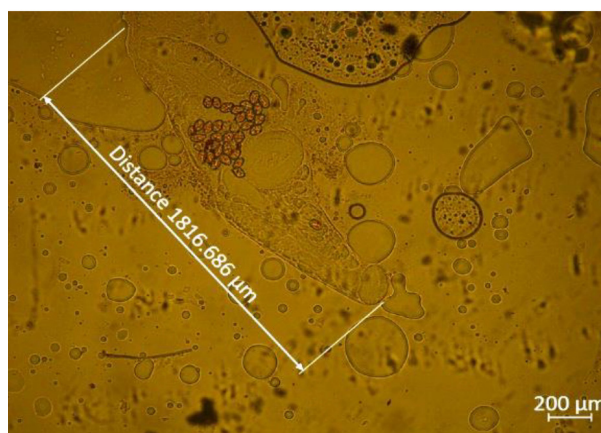
## Results

During the parasitological investigations on *Squalius squalus* from Belčišta Wetland, six parasite species were found: *Dactylogyrus sphyrna* von Linstow,

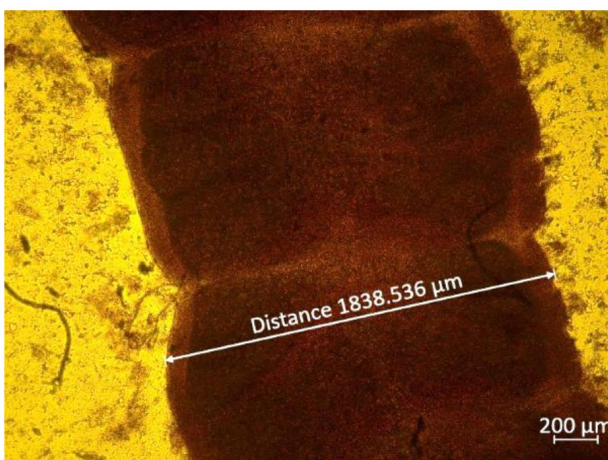




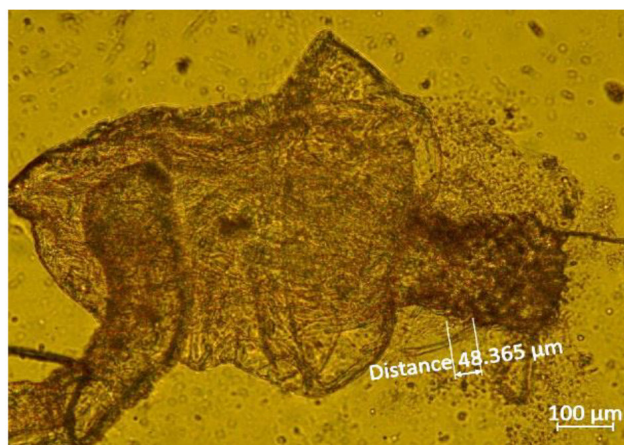
**Fig. 5.** *Paradiplazoon ergensi* (Pejčoch, 1968) clamps.



**Fig. 6.** *Allocreadium isoporum* (Looss, 1894), a native specimen in the intestinal contents.



**Fig. 7.** *Proteocephalus torulosus* (Batsch, 1786), scolex (left) and uterine proglottides (right).



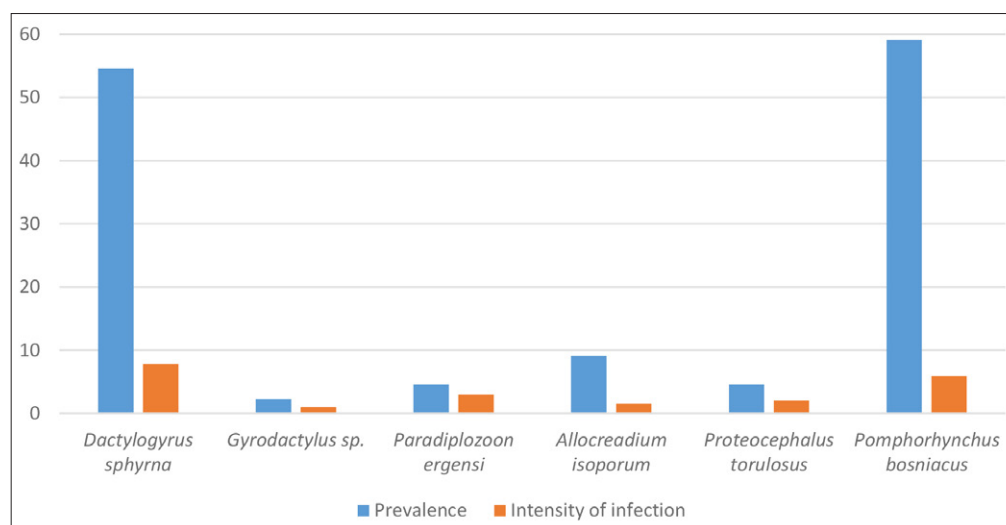
**Fig. 8.** *Pomphorhynchus bosniacus* Kiskároly and Čanković, 1969, specimens in the intestine (left) and a specimen with protruded proboscis (right).

1878 (Fig. 3), *Gyrodactylus* sp. (Fig. 4), *Paradiplazoon ergensi* (Pejčoch, 1968) (Fig. 5), *Allocreadium isoporum* (Looss, 1894) (Fig. 6), *Proteocephalus torulosus* (Batsch, 1786) (Fig. 7) and *Pomphorhynchus bosniacus* Kiskároly and Čanković, 1969 (Fig. 8).

The total prevalence of infection with helminths was 90.91 %, i.e. 40 out of 44 examined fish specimens were infected. The most prevalent parasites were *P. bosniacus* (59.09 %) and *D. sphyryna* (54.55 %), while the least prevalent was *Gyrodactylus*

**Table 1.** Infection characteristics of the parasites of the chub *Squalius squalus* from Belčišta Wetland based on the examination of 44 fish specimens.

Parasite species	No. of infected fishes	Prevalence (%)	Mean intensity
<i>Dactylogyrus sphyrna</i>	24	54.55	7.83
<i>Gyrodactylus</i> sp.	1	2.27	1.0
<i>Paradiplozoon ergensi</i>	2	4.55	3.0
<i>Allocreadium isoporum</i>	4	9.09	1.50
<i>Proteocephalus torulosus</i>	2	4.55	2.0
<i>Pomphorhynchus bosniacus</i>	26	59.09	5.92
All helminth parasites	40	90.91	6.08

**Fig. 9.** Parasite fauna of the chub *Squalius squalus* from Belčišta Wetland.

*lus* sp. (2.27 %) (Table 1, Fig. 9). The total mean intensity of infection was 6.08, with maximum values for *D. sphyrna* (7.83) and *P. bosniacus* (5.92) while *Gyrodactylus* sp. had the lowest mean intensity (1.0) (Table 1, Fig. 9).

## Discussion

The parasite fauna of the chub from Belčišta Wetland is in common with that of the fishes of the family Cyprinidae from the Balkan Peninsula and adjacent areas (Ergens 1960, 1970, Chankovic et al. 1968, Kakacheva-Avramova, 1983, Hristovski 1983, Bauer 1985, 1987, Dupont & Lambert 1986, Nedeva-Lebenova 1991, Cakic, 1992, Djikanovic et al. 2012, Stojanovski 1997, 2003, Stojanovski et al. 2012, Blazhekovikj-Dimovska et al. 2023). Some of the recorded parasites have a wide geographical distribution and wide host ranges, e.g. *Allocreadium isoporum* and *Proteocephalus torulosus*. The findings of *Dactylogyrus sphyrna*, *Paradiplozoon ergensi*, *Gyrodactylus* sp., *Allocreadium isoporum* and *Proteocephalus torulosus* represent the first record

for *Squalius squalus* from Belčišta Wetland. The record of *Proteocephalus torulosus* in *Squalius squalus* is the first observation of this parasite in this host species in the Republic of North Macedonia.

Among the parasite species recorded in chub from Belčišta Wetland, the species that are frequently reported as fish pathogens are *Dactylogyrus sphyrna*, *Paradiplozoon ergensi* and *Pomphorhynchus bosniacus*.

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