

FIRST RECORD OF *POMPHORHYNCHUS BOSNIACUS* (KISKÁROLY ET ČANKOVIĆ, 1969) (PALEACANTHOCEPHALA: POMPHORHYNCHIDAE) IN SOME CYPRINID FISH FROM BELČIŠTA WETLAND (MACEDONIA)

Dijana Blazhekovikj-Dimovska^{1*}, Stojmir Stojanovski², Lidija Velkova-Jordanovska¹, Vasil Kostov³, Irina Manevska³, Julijana Arsovska⁴, Kristijan Veljanovski¹

- ¹University “St. Kliment Ohridski”, Faculty of Biotechnical Sciences, “Partizanska” b.b. 7000 Bitola, N. Macedonia;
²Hidrobiological Institute, “NaumOhridski”50, 6000 Ohrid, N. Macedonia;
³University "St. Cyril and Methodius", Skopje, Institute of Animal Sciences
⁴University "St. Cyril and Methodius", Skopje, Faculty of Natural Sciences and Mathematics, Institute of Biology

Abstract: The representatives of the phylum Acanthocephala are of great importance in fish pathology. *Pomphorhynchus bosniacus* (Kiskároly et Čanković, 1969) (Paleacanthocephala: Pomphorhynchidae) is an intestinal parasite of many freshwater fishes confined to limited geographical areas of southeastern Europe (waters of Balkan), including Bosnia and Herzegovina and N. Macedonia. In our case study, a total of 33 specimens of 3 cyprinid fish (*Squalius squalus*, *Phoxinus lumaireul* and *Pelaspus minutus*) from Belčišta wetland (southwest N. Macedonia) were subjected to a parasitological investigation, by seasons. Only fresh fish were subjected to routine identification, dissection, and observation methods. Parasite identification was performed morphologically, based on the character of proboscis with hooks and the reproductive system, using referent key for determination. Thereby, infestation by the parasite was determined in all fish species, so the prevalence of infestation is the highest in *Phoxinus lumaireul*(80.0%), then *Pelaspus minutus* (75.0%) and the lowest is in *Squalius squalus* (66.67%).Infestation intensity is the highest in *Squalius squalus* (5.40), then *Phoxinus lumaireul* (4.75), and the lowest is in *Pelaspus minutus* (3.0).This parasite species can lead to heavy impairing of fish health status because the parasite penetrates the bulb and proboscis through the gut wall into the peritoneal lumen and even attach on abdominal organs. The record of *Pomphorhynchus bosniacus* in all 3 cyprinid fish in the present study is considered as the first in Belčišta wetland. At the same time, *Phoxinus lumaireul* and *Pelaspus minutus* represent new hosts for this parasite worldwide.

Keywords: *Pomphorhynchus bosniacus*, cyprinid fish, Belčišta wetland, first record

INTRODUCTION

Belčišta wetland (also called Sini Viroj) is located in the municipality of Debrca, below Ilinska Mountain, at an altitude of about 767 m. It is a remnant of the former Desaret Lake, which flooded the Debrca valley in the Pliocene. The wetlandis fed with 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 surface of the wetland covers around 400 hectares. There are also several wetland lakes in the wetland, 3 of which are larger.With the retreat of the Desaret Lake along the Sateska River, numerous endemic species of plant and animal life continued to exist in the waters of the wetland. Belčišta wetland is the largest and one of the most important wetlands in N. Macedonia, with flooded forests and wet pastures, because it is well preserved. Specific is also due to different types of wetland habitats, especially flooded alder forests. There is a great diversity of flora and fauna. Belčišta wetlandis part of the Emerald National Network of the Republic N. Macedonia, and is proposed to be nominated as a Natura 2000 location. The acanthocephalans in genus *Pomphorhynchus* are obligate parasites of freshwater fish and, less frequently, of marine fish, amphibians and mammals. The genus *Pomphorhynchus* include 32 species distributed over all continents and six of which have been described from the European continent As Pomphorhynchidae in general exhibit only few differentiating morphological characters and show a high intraspecific variability, species identification based on morphological characters alone remains challenging. Due to the morphological similarity between different species from the genus *Pomphorhynchus* they might have been repeatedly misidentified, which possibly led to an underestimation of the distribution and abundance of each species throughout Europe. The pathogenicity of Acanthocephala is caused by two main factors: density of parasites and depth of parasite penetration. Also, patterns of hook arrangement, as well as various morphological features such as presence of the bulb can enhance the extent of tissues damage. Most parasitic organisms are in a co-evolutionary arms race with their host species. In horizontally transmitted endoparasites this generally results in relatively low parasitic virulence for the parasites’ adult stages in the final host. Additionally, invasive species can contribute to the life cycle of native parasites. If an invasive species can serve as a suitable (e.g. intermediate, paratenic or final) host for local parasites, these parasites may “spill back” to other local hosts which will consequently increase their infection rates within the native host populations in newly invaded habitats. For example, gobies in the Rhine River can contribute to Asian swim bladder nematode *Anguillicoloides crassus* colonizing the European eel (*Anguilla anguilla*), by serving as a paratenic host, thereby leading to higher infection levels in its definitive hosts, the European eel. In contrast, invasive species might also be responsible for a decrease of the infection risk in the native host populations. Globalization, the transfer and invasion of non-indigenous species, has caused widespread biotic homogenization and the replacement of local species, resulting in a worldwide biodiversity loss. Several mechanisms, such as different environmental tolerance, higher reproduction rates, or different aggression and mutual predation can be involved in the regulation of the competitive interactions between native and non-indigenous species. Following the replacement of the native fauna, nonindigenous species can transform habitats and even threaten entire ecosystems. They can alter ecosystem processes, causing serious problems to the environment and major economic losses.

P. bosniacus can also use the alternative transmission route via a paratenic host. Suitable definitive host for acanthocephalans of the genus *Pomphorhynchus* in the investigated section of the Rhine River are mostly cyprinids such as chub (*Squalius cephalus*), common barbel (*Barbus barbus*) and idle (*Leuciscus idus*). Small fish might become one of the most important food items in the diet of large chubs or common barbel. Therefore, it can be assumed that after their establishment in newly invaded habitats the gobies might become an essential part of the diet of piscivorous and probably some omnivorous fish species, as reported for pikeperch from the newly colonized Kiel Canal or for pikeperch and perch from western Baltic Sea. Thus, gobies are most probably also preyed as food source by different large cyprinids and might enhance the transmission of *Pomphorhynchus* species in their area of distribution.

MATERIAL AND METHODS

Fish material from a total of 33 specimens of 3 cyprinid fish from Belčišta wetland (southwest N. Macedonia) were subjected to a parasitological investigation, by seasons this year. Only fresh fish 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). For the collection of acanthocephalan species, intestines of fish were examined using the stereomicroscope “Zeiss Stemi 305” and microscope “Zeiss Primovert” and parasites were removed. For morphological examination, permanent slide of whole individual parasite was prepared by staining with acetocarmine, dehydrating with ascending grades of alcohol and mounting in Canada balsam. Identification was made throughout the morphology of neck, bulb, proboscis with hooks and the reproductive system, using referent key for determination (Bauer, 1985; Čanković et al., 1968).

Table 1. Infestation of cyprinid fish from Belčišta wetland with *Pomphorhynchus bosniacus*.

Fish species	Season											
	Winter			Spring			Summer			Autumn		
	Examined	Infested		Examined	Infested		Examined	Infested		Examined	Infested	
		Prevalence (No. and %)	Intensi ty		Prevalence(No. and %)	Intensity		Prevalence(No. and %)	Intensity		Prevalence(No. and %)	Intensity
<i>Squalius squalus</i>	1	1 (100%)	6.0	5	2 (40%)	10.0	3	3 (100%)	3.67	6	4 (66.67%)	4.50
<i>Phoxinus lumaireul</i>	0	0	0	0	0	0	0	0	0	10	8 (80%)	4.37
<i>Pelaspus minutus</i>	1	0	0	0	0	0	0	0	0	7	6 (85,71%)	3.17
IN TOTAL	2	1 (50%)	6.0	5	2 (40%)	10.0	3	3 (100%)	3.67	23	18 (78.26%)	4.0

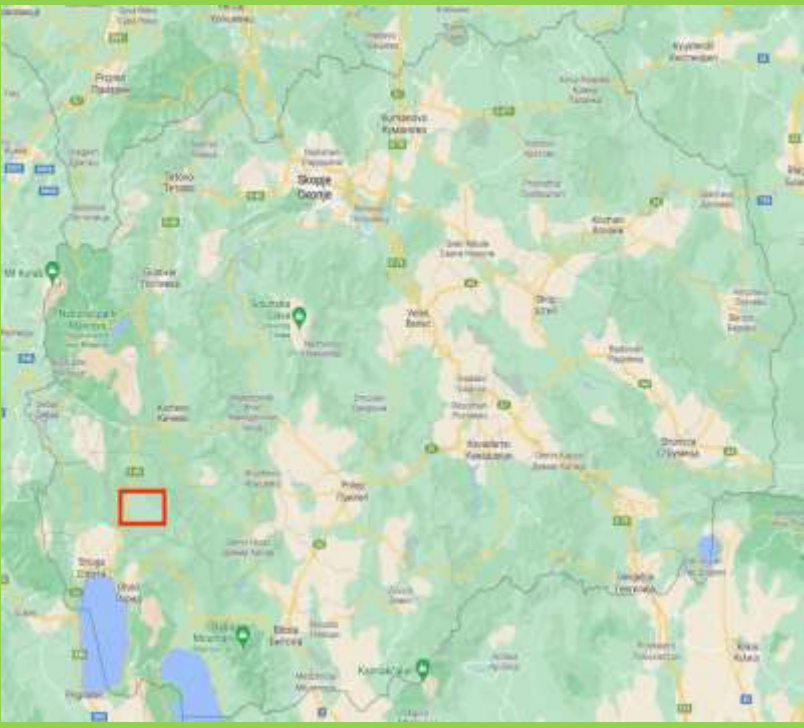


Fig. 4. *Pomphorhynchus bosniacus* from minnow (*Phoxinus lumaireul*) from Belčišta wetland - proboscis of a female worm with bulb and hooks – original photo.



Fig. 5. *Pomphorhynchus bosniacus* from chub (*Squalius squalus*) from Belčišta wetland - proboscis of a female worm with hooks – original photo.

RESULTS AND DISCUSSION

During the parasitological investigations of the cyprinid fish from Belčišta wetland, from a total of 33 fish samples examined, parasites *Pomphorhynchus bosniacus* were found in 24 fish (72.73%). Three cyprinid fish species that were examined and all of them were infested: chub - *Squalius squalus* (Bonaparte, 1837) – 15 specimens, minnow – *Phoxinus lumaireul* (Schinz, 1840) and Ohrid’ grunče – *Pelaspus minutus* (Karaman, 1924). The total intensity of infestation was 4.54 (Table 1).

Chub (*Squalius squalus*) - from 15 fish examined, 10 or 66.67% were infested with adult specimens of *P. bosniacus*. Intensity of infestation at chub was 5.50 (Table 1, Fig. 3).

Phyllum ACANTHOCEPHALA
Class PALEACANTHOCEPHALA
Ordo ECHINORHYNCHIDA
Family POMPHORHYNCHIDAE
Ordo POMPHORHYNCHUS
Species *POMPHORHYNCHUS BOSNIACUS* (Kiskároly et Čanković, 1969)

This parasite species was found in adult stage in the intestines of *Squalius squalus* and in subadult stage in the body cavity of *Phoxinus lumaireul* and *Pelaspus minutus*. *Pomphorhynchus bosniacus* is determined morphologically throughout observation on the shape and measurements of neck, bulb, proboscis with hooks and the reproductive system. Although the specimens of this study were slightly smaller in size than *P. bosniacus* described by Kiskároly and Čanković (1969), the size and arrangement of the hooks, the length of the neck, bulb and proboscis with hooks (Kiskároly and Čanković, 1969; Moravec, 2004) suggest that the specimens of the Belčišta wetland belong to the same species. According to Bauer (1985), intermediate hosts for the representatives of the genus *Pomphorhynchus* are gammarids, and in Belčišta wetland have been already found *Gammarus roeselii* and *G. balcanicus* (Zoroski, 2022).

The distribution of this parasite is limited to the Balkan Peninsula, especially its western part, as well as the Danube river basin (Hristovski, 1983, Stojanovski, 1997; Reier et al., 2019). The acanthocephalan *P. bosniacus* was first described at *Barbus barbus* (L.) from the River Sava in Bosnia and Herzegovina as nomen nudum (Kiskároly & Čanković, 1967), followed by differential diagnosis in 1969 (Kiskároly & Čanković, 1969).

The latter species was reported from various other fishes from lakes and rivers of the Balkan: Djikanovic et al. (2012), Kažić (1970), Kakacheva-Avramova (1973), Nedić et al. (2019) According to the data of Hristovski (1975, 1983) and Stojanovski (1997) *P. bosniacus* was found in N. Macedonia at: *Salmo letnica*, *S. ohridana*, *S. peristericus*, *Anguilla anguilla*, *Alburnus scoranza*, *Rutilus ohridanus*, *R. prespensis*, *Pachychilon pictum*, *Squalius squalus*, *Cyprinus carpio*, *Scardinius knezevici*, *Barbus petenyi*, *B. prespensis* and *Gobio ohridanus*.

The finding of *Pomphorhynchus bosniacus* in cyprinid fish in the present study is in line with literature data. The record of this parasite is considered as the first in N. Macedonia. At the same time, *Phoxinus lumaireul* and *Pelaspus minutus* represent new hosts for this parasite worldwide. Although *P. bosniacus* has a smaller proboscis with a lower number of hooks compared to *P. laevis*, they both have a bulb, the key feature that fortifies the “rivet-like mode” of attachment to the host intestine. There action can be caused by the parasite trunk free in the gut lumen; by the embedded parasite neck in the gut wall; by the parasite bulb and proboscis localised in the intestinal wall; and by penetration of bulb and proboscis into the liver and pancreas. Having a longer neck compared to *P. laevis*, only a small anterior part of *P. bosniacus* body is embedded in the intestinal wall together with the neck. Haemorrhages and inflammatory changes caused by the neck in the tunica mucosa, submucosa and muscular layer are in line to those previously described (Dezfuli et al., 2002).

In our case, similar pathological changes caused by *P. bosniacus* were found, during which parasites completely pierced the intestinal wall with their scolex, and they enter the body cavity with their bulb and proboscis. Consequently, secondary bacterial infections that cause more severe inflammatory processes, intoxications and mass deaths can occur.

During the investigations that follow, we expect to find more species of parasites in the fish in the Belčišta wetland, some of which will certainly be new to the parasite fauna of fish from N. Macedonia, and this will contribute to supplementing knowledge and obtaining a more complete picture of the pathology of fish in N. Macedonia, but also the measures that would be taken to prevent the diseases.

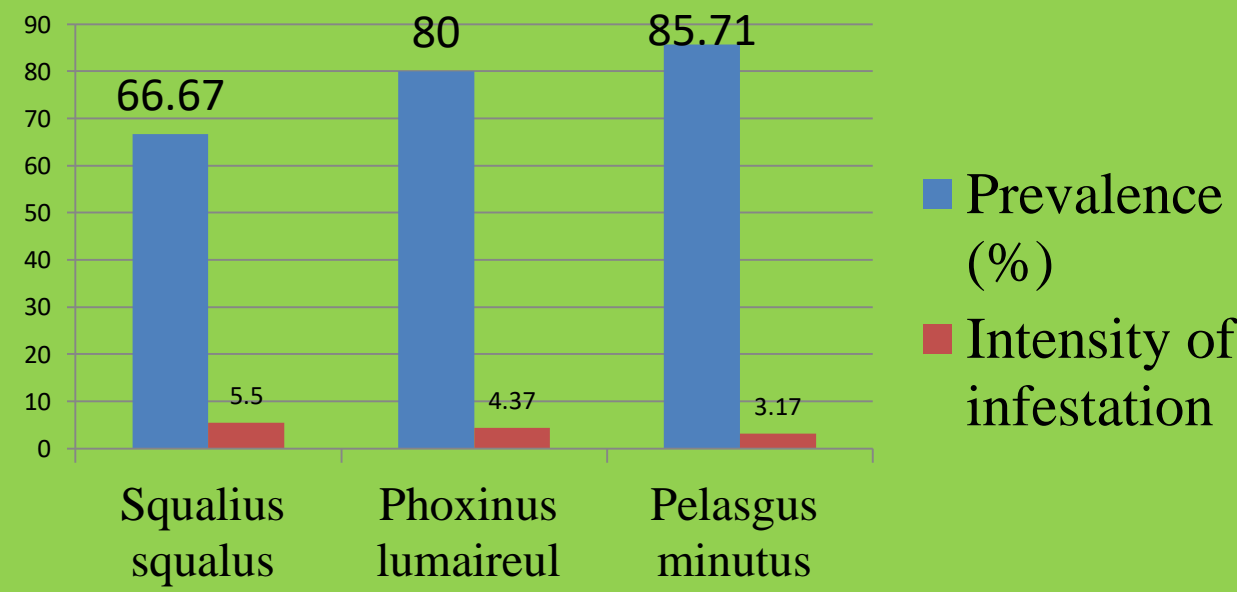


Fig. 3. Prevalence and intensity of infestation of cyprinid fish from Belčišta wetland with *Pomphorhynchus bosniacus*.