

HELMINTH PARASITES OF TWO CYPRINID FISHES FROM TOPOLNITSA RIVER, BULGARIA

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Abstract

During summer 2019, 15 specimens of round-scaled barbell (*Barbus cyclolepis* Heckel, 1837) and 12 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) from Topolnitsa River were collected and examined with standard techniques for parasites. Helminth parasites were recorded in 9 round-scaled barbell specimens (60%) and 7 Orpheus dace specimens (58.33%) from Topolnitsa River. Only one parasite species was identified in both cyprinid fishes – the acanthocephalan species *Pomphorhynchus laevis* (Zoega in Müller, 1776). Bioindicator significance of established parasite species was discussed for ecological evaluation of the state of the studied freshwater ecosystem. New data for the helminths of round-scaled barbell and Orpheus dace from Topolnitsa River are presented.

Key words: *Barbus cyclolepis*, helminths, *Squalius orpheus*, Topolnitsa River.

INTRODUCTION

Topolnitsa River is a river in Southern Bulgaria, left tributary of the Maritsa River. With a length of 154.8 km, Topolnitsa River is one of the longest rivers in the Maritsa catchment area. Topolnitsa River is one of the heavily polluted rivers in Bulgaria (Zhelev et al., 2013). Species composition of the ichthyofauna, heavy metal and toxic element levels in aquatic mosses, aquatic macrophytes and sediments, changes in the morphological content of the blood of amphibian species as a result of anthropogenic pollution of Topolnitsa River and its basin were studied (Yurukova and Gecheva, 2012; Gecheva et al., 2013; Kolev, 2013; Zhelev et al., 2013). Species composition of the ichthyofauna of Topolnitsa River is represented by 13 fish species belonging to 4 families (Kolev, 2013). In Topolnitsa River, two species belonging to Cyprinidae family predominate - *Barbus cyclolepis* and *Squalius orpheus* (Kolev, 2013). The area of the Topolnitsa River has been subject to constant heavy metal pollution for many years (Velcheva and Nikolov, 2009). Parasites are indicators for the ecosystem stability, biodiversity and environmental health (Marcogliese and Price, 1997; Marcogliese, 2003; 2004; 2005; Sures et al., 2017). This

study aims to present the diversity of parasites of round-scaled barbell and Orpheus dace from Topolnitsa River. As a result of this survey, new data for helminths of *B. cyclolepis* and *Sq. orpheus* is presented.

MATERIALS AND METHODS

In early summer of 2019 fish and fish parasites were collected and examined from Topolnitsa River (village Yunatsite). The village Yunatsite is located on the south bank of Topolnitsa River. The River springs from the Sredna Gora Mountain in central Bulgaria. Almost the entire Topolnitsa River from the springs to above the confluence of the Mativir River is a Protected Zone (NATURA 2000 zones: Sredna Gora BG0001389).

A total of 15 specimens of the round-scaled barbell (*Barbus cyclolepis* Heckel, 1837) and 12 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) from Topolnitsa River were collected and examined in 2019. Fish were caught by angling. The scientific and common names of fish hosts are used according to the FishBase database (Fröse and Pauly, 2020).

The fish samples were examined immediately after their capture for gastrointestinal parasites using standard techniques. The samples are

counted and identified using keys of Bauer et al. (1981), Bauer (1987) and Bykhovskaya-Pavlovskaya (1985). Acanthocephalans are examined as temporary slides in ethanol-glycerin and identified (Petrochenko, 1956; Ergens and Lom, 1970; Bykhovskaya-Pavlovskaya, 1985).

The ecological terms prevalence, mean intensity (MI) and mean abundance (MA) are used and calculated, based on Bush et al. (1997). The dominant structure of the component helminth communities is determined according to the criteria proposed by Kennedy (1993) based on the prevalence (P%) as: accidental (P% < 10), component (P% < 20) and core (P% > 20) species.

RESULTS AND DISCUSSIONS

A total of 15 specimens of the round-scaled barbell (*Barbus cyclolepis* Heckel, 1837) and 12 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) from Topolnitsa River are collected and examined in 2019. The round-scaled barbell and Orpheus dace are not included in the Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011). *Barbus cyclolepis* and *Squalius orpheus* are estimated as least concern species (LC=Least Concern; IUCN Red List Status). Round-scaled barbell is freshwater, benthopelagic fish species (Fröse and Pauly, 2020). This fish species inhabits upper and

middle reaches of flowing rivers with sandy-gravelly bottom. *B. cyclolepis* feeds on larvae of chironomids, caddisflies, mayflies and plant detritus (Karapetkova and Zhivkov, 2006). *B. cyclolepis* is endemic fish to the Maritsa River Basin (Kolev, 2013).

Orpheus dace is freshwater, pelagic, omnivorous fish species. Young fish feeds on algae and crustaceans, and adults - insects and their larvae, fish, frogs and small rodents (Karapetkova and Zhivkov, 2006). This fish species occurs in small to larger streams and rivers with standing water to moderate current (Fröse and Pauly, 2020). *Sq. orpheus* is endemic fish to the Aegean Basin (Kolev, 2013).

A total of 15 specimens of the round-scaled barbell (*Barbus cyclolepis* Heckel, 1837) and 12 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) from Topolnitsa River were collected and examined for parasites. Helminth parasites were recorded in 9 round-scaled barbell specimens (60%) and 7 Orpheus dace specimens (58.33%) from Topolnitsa River. Only one parasite species was identified in both cyprinid fishes – the acanthocephalan species *Pomphorhynchus laevis* (Zoega in Müller, 1776) (Table 1). The only established helminth species occurred as an adult. *P. laevis* is autogenic species, matured in fish. The studied two cyprinid fish hosts showed similar prevalence, mean abundance and mean intensity (Table 1).

Table 1. Ecological indices of the helminth parasites of *B. cyclolepis* and *Sq. orpheus* from Topolnitsa River (N - number of examined fish specimens, n - number of infected hosts, p - number of parasites, P% - prevalence, MA - mean abundance, MI - mean intensity)

Host	Helminth species	N	n	p	P%	MA±SD	MI±SD	Range
<i>Barbus cyclolepis</i>		15	9	27	60.00	1.8±2.34	3.0±2.36	1-8
	<i>Pomphorhynchus laevis</i>							
<i>Squalius orpheus</i>		12	7	21	58.33	1.75±3.19	3.0±3.70	1-12
	<i>Pomphorhynchus laevis</i>							

The established in this study *Pomphorhynchus laevis* is an intestinal parasite of many freshwater fish, most often by a family Cyprinidae and less frequently by other families (Kakacheva-Avramova, 1983). The life cycle of *P. laevis* is accomplished with the participation of the intermediate host

Gammarus pulex (Amphipoda), and definitive host - fish (Petrochenko, 1956). *G. pulex* is a bioindicator for β-mesosaprobity (Johnson et al., 1993).

P. laevis was reported as a parasite of *B. cyclolepis* from Tundzha River (Kakacheva-Avramova, 1972). For Bulgaria, *C. brachycollis*, *C. fennica*, *Caryophyllaeides*

sp. juv., *B. rectangulum*, *Cestoidea* g. sp., *Allocreadium isoporum*, *Allocreadium isoporum macrorchis*, *Diplostomum spathaceum* larv., *Dactylogyrus carpathicus*, *D. dyki*, *D. petenyi*, *Gyrodactylus albaniensis*, *G. markewitschi*, *Paradiplozoon homoion*, *Diplozoon* sp., *Rhabdochona gnedini*

(=*Rhabdochona sulaki*), *R. denudata*, *Rhabdochona hellichi*, *Schulmanella petruschewskii* (= *Capillaria petruschewskii*), *Nematoda* gen. sp., *Acanthocephalus anguillae*, *Neoechinorhynchus rutili* and *P. laevis* were reported as parasites of *B. cyclolepis* (Table. 2).

Table 2. Overview of helminth species of *Barbus cyclolepis* registered in Bulgaria

Authority	Margaritov (1965)	Kakacheva-Avramova (1965)	Kakacheva-Avramova (1972)	Kirin (2002a)	Kirin (2003)	This study
Helminth species						
Cestoda						
<i>Caryophyllaeus brachycollis</i>	•	•	•	•	•	
<i>Caryophyllaeides fennica</i>	•	•	•		•	
<i>Caryophyllaeides</i> sp. juv.		•				
<i>Bathybothrium rectangulum</i>	•	•		•	•	
<i>Cestoidea</i> g.sp.	•					
Trematoda						
<i>Allocreadium isoporum</i>	•	•	•**			
<i>Allocreadium isoporum macrorchis</i>				•		
<i>Diplostomum spathaceum</i> larv.		•				
<i>Dactylogyrus carpathicus</i>			•			
<i>Dactylogyrus dyki</i>			•			
<i>Dactylogyrus petenyi</i>		•				
<i>Gyrodactylus albaniensis</i>		•				
<i>Gyrodactylus markewitschi</i>			•			
<i>Paradiplozoon homoion</i>			•			
<i>Diplozoon</i> sp.		•*				
Nematoda						
<i>Rhabdochona gnedini</i>	•					
<i>Rhabdochona denudata</i>	•	•				
<i>Rhabdochona hellichi</i>					•	
<i>Schulmanella petruschewskii</i>		•				
<i>Schulmanella</i> sp.			•			
<i>Nematoda</i> g.sp.	•					
Acanthocephala						
<i>Acanthocephalus anguillae</i>	•			•	•	
<i>Neoechinorhynchus rutili</i>		•			•	
<i>Pomphorhynchus laevis</i>			•			•

* reported from Kakacheva-Avramova (1965) as *Diplozoon* sp. and *Diplozoon* sp. diporpa

** reported from Kakacheva-Avramova (1972) as *Allocreadium isoporum isoporum*

The parasite fauna of *B. cyclolepis* from River Topolnitsa was studied from Margaritov (1965) and Kakacheva-Avramova (1965). Margaritov (1965) studied the parasite fauna of *B. cyclolepis* from the middle reaches of River Maritsa and its tributaries - Chepinska, Topolnitsa and Vacha rivers. For Topolnitsa River, the author established for round-scaled

barbell four parasite species: *Caryophyllaeus brachycollis*, *Caryophyllaeides fennica*, *Rhabdochona denudata* and *Nematoda* g. sp. Margaritov (1965) specifically notes that acanthocephalan species has not been revealed in fish from Topolnitsa River. Kakacheva-Avramova (1965) reported *C. fennica* and *C. brachycollis* for *B. cyclolepis* from the rivers

Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijska, Sushenitsa and Bedechka. The author also reported *Caryophyllaeus* sp. juv. of *B. cyclolepis*, from the Maritsa, Chepinska and Harmanlijska rivers.

Rozdina et al. (2008) studied the food spectrum and feeding of *Barbus cyclolepis* from the middle stream of the Maritsa River (Bulgaria). According to them, the food of *B. cyclolepis* is dominated by Chironomid larvae, followed by plant detritus and Gamarids.

Kakacheva-Avramova (1965) investigated the parasite fauna of *Sq. orphaeus* (reported as *Leuciscus cephalus*) from water basins in Trakia (southern Bulgaria). The author reported *C. fennica* of *L. cephalus* from the rivers Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijska, Sushenitsa and Bedechka.

Kakacheva-Avramova (1965) also reported *C. brachycollis* for *L. cephalus* from the rivers Asenitsa, Sjujutlijska, Chepinska, Bedechka and Topolnitsa. The author also reported *Caryophyllaeus* sp. of *L. cephalus* from the rivers Maritsa, Syuyutlijska and Harmanlijska. In most of the conducted studies of the parasite communities of the Orpheus dace from The Maritsa River Basin *Pomphorhynchus laevis* has been reported, including and in this study (Table 3). Only Kakacheva-Avramova (1965) did not report any acanthocephalan species. The number of helminth species reported for the helminth communities of *Sq. orphaeus* from The Maritsa River Basin ranged from 1 (this study) to 8 (Kirin, 2002b; Kirin et al., 2005) (Table 3).

Table 3. Overview of helminth species of *Squalius orpheus* registered in Maritsa River Basin, Bulgaria

Authority Helminth species	Margaritov (1965)	Kakacheva- Avramova (1965)	Kirin (2000a)	Kirin (2000b)	Kirin (2001)	Kirin (2002b)	Kirin et al. (2005)	Kirin et al. (2019)	This study
Cestoda									
<i>Caryophyllaeus brachycollis</i>	•	•	•	•	•		•	•	
<i>Caryophyllaeides fennica</i>	•	•					•		
<i>Bathybothrium rectangulum</i>			•	•	•	•	•		
Trematoda									
<i>Allocreadium isoporom</i>	•	•						•	
<i>Allocreadium isoporom macrorchis</i>			•	•	•		•		
<i>Clinostomum complanatum</i>			•	•	•				
<i>Dactylogyrus parvus</i>		•							
Nematoda									
<i>Nematoda</i> sp.	•*								
<i>Contracaecum</i> sp.						•**			
<i>Rhabdochona denudata</i>	•	•				•	•	•	
<i>Philometra abdominalis</i>			•	•					
Acanthocephala									
<i>Acanthocephalus anguillae</i>	•			•	•	•	•		
<i>Acanthocephalus tenuirostris</i>			•	•		•	•		
<i>Pomphorhynchus laevis</i>			•	•	•		•	•	•

•*reported from Margaritov (1965) as *Agamonema* sp. larva I

•**reported from Kirin (2002b) as *Contracaecum squalii* (see Moravec, 2013)

CONCLUSIONS

River Topolnitsa is a new locality of *Pomphorhynchus laevis*. The determined helminth species *P. laevis* is a core species for the helminth communities of *B. cyclolepis* and *Sq. orpheus* from the studied ecosystem.

The establishment of only one intestinal parasite in both fish hosts indicated poor species diversity within the studied freshwater habitat and negative impacts on the ecosystem.

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