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HELMINTS AND HELMINTH COMMUNITIES OF *Squalius cephalus* (Linnaeus, 1758) FROM OSYM RIVER, BULGARIA

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Abstract

During 2018, the first ecologoparasitologycal study of Squalius cephalus from Osym River, a tributary of Danube River, Bulgaria was made. In 20 specimens of examined common chub, 3 species of endohelminths are established (Ichtyocotylurus pileatus (Rudolphi, 1802) Dubois, 1937 Metacercaria; Caryophyllaeus brachycollis Janiszewska, 1951; Rhabdochona denudata (Dujardin, 1845) Raillet, 1916). C. brachycollis and Rh. denudata are autogenic species, whereas I. pileatus isallogenic species. I. pileatus is reported for the first time for the freshwater fish fauna of Osym River. Sq. cephalusis a new host record for I. pileatus in Bulgaria. The basic ecological characteristics and biotic indices of the parasite populations and communities are determined. The dominant structure of the endohelminth communities is presented on the level of the component community.

Key words*: helminths, helminth communities, Osym River, Squalius cephalus.*

INTRODUCTION

River Osam refers to Type R7: Large tributaries of the Danube River in Ecoregion 12 (Pontian province). The freshwater ecosystem is a subject of impact monitoring as a part of the National Environmental Monitoring System (Belkinova et al., 2013; Peev and Gerassimov, 1999). The river ecosystem and its adjacent territories are characterized by great biodiversity. This is the reason for the announcement of the protected areas BG 0000615 Devetashko Plato and BG 0000616 Mikre, associated with the river ecosystem (Directive 92/43; Natura 2000), etc. The fish fauna of the OsamRiverhas been studied by a number of authors (Vassilev and Pehlivanov, 2005; Zarev et al., 2013). At the same time, no studies on parasites and parasite communities of *Squalius cephalus* (Linnaeus, 1758) of the OsamRiverhave been conducted. Parasites are interesting as bioindicators of different biological aspects of fish host's and for environmental quality status (Marcogliese and Cone, 1997; Galli et al., 2001; Tieri et al., 2006). The complex biological cycles of endoparasites reflect the relationships with a number of invertebrate and vertebrate hosts. The species diversity of parasites and characteristics of the endoparasite communities indicated a seasonal variation of water characteristics and state of biodiversity in the environment (Tieri et al., 2006; Lamková et al., 2007). The aim of the study is to present the results from the examinations of the endoparasite species, as well as to study the ecological characteristics of the helminth communities of *Sq. cephalus* of the Osam River (Danube Basin).

MATERIALS AND METHODS

The River Osam takes it source from Levskipeak (1,821 m above sea level), Balkan mountain and flows into the Danube River, not far away from the town of Nikopol (5 km; 40 m above sea level).The river is 314 km long with 2,838 km^2 size of the basin. The studied specimens of chub are collected by angling during 2018 in the vicinity of the town of Lovech ($43^0$08'05"N $24^0$43'02"E; north-central Bulgaria).Town of Lovech is divided into two parts of the Osam River (Statistical Yearbook, 2017). The river is broad, mainly with a slow stream. The river be disband with organic sediments, clay, etc., where it is distinguished by pebble and rocky areas (Belkinova et al., 2013). The water in the upper part of the river is used for electricity and in the middle and lower part for irrigation and for industry

(Statistical Yearbook, 2017).A total of 20 specimens *Squalius cephalus* (Linnaeus, 1758) are examined for endohelminths. The scientific and common names of the fish are presented according to the FishBase database (Fröse and Pauly, 2018). Helminthological examinations are carriedout following recommendations described by Zashev and Margaritov (1966); Byhovskaya-Pavlovskaya (1985); Bauer (1987); Protasova et al. (1990); Moravec (2013). Specimens are fixed and preserved in 70% ethyl alcohol.The specimens of Trematoda and Cestoda are studied by methods of Georgiev et al. (1986); Scholz and Hanzelová (1998). The nematodes are studied on temporary mounts with 5% glycerolin 70% ethanol (Zashev and Margaritov, 1966; Moravec, 2013). Analyses of helminth community structureare carried out in both levels: infracommunity (total number of species; total and mean number of specimens; Brillouin's index of diversity (HB); Pielou index of evenness (E)) and component community (prevalence (P%) and mean intensity (MI) for each species) (Bush et al., 1997; Magurran, 1988).The species are divided into core species (P%>20), component species (P%>10) and accidental species (P%<10) (Kennedy,1997; 1993). The diversity measures are calculated by software products Statistica 10 (StatSoft Inc., 2011) and MS Excel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Fish communities

During 2018, a total of 20 specimens of chub (*Squalius cephalus* (Linnaeus, 1758); Cyprinidae) are examined for endohelminths. *Sq. cephalus*is included in the list of IUCNas least concern species (LC=Least Concern; IUCN Red List Status, 2018). The chub is not included in the Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011). The chub inhabits slow to-medium-flowing waters in the lower and middle streams of rivers, irrigation canals, reservoirs. The species is distinguished by specific migrations, pursuing small passages of fish, for example, which in autumn, in periods of low water, leave the shallows and go to greater depths for greater security. The species prefer habitats to steep, steeply descending coasts, as well as pebbles or sandy bottom. *Sq. cephalus* is an omnivorous species. It feeds on small fish, small frogs, crabs and even mice. The chub is predominantly with daily activity, but during the hottest periods of the year shifts its demand for food early in the morning and at night. The species is characterized by year-round activity (Karapetkova and Zhivkov, 2006; Fröse and Pauly, 2018).

Helminth community structure

During 2018, 20 specimens of chub are infected with 3 species of endoparasites, belonging to the classes Trematoda, Cestoda and Nematoda. They are *Ichtyocotylurus pileatus* (Rudolphi, 1802) Dubois, 1937 (metacercaria); *Caryophyllaeus brachycollis (*Janiszewska, 1951) and *Rhabdochona denudata* (Dujardin, 1845; Raillet, 1916). A total, 28 specimens of parasites are studied. Matures of *I. pileatus* have a site of infection at intestines and a factory bag of seagulls. They are specific definitive hosts of these parasite species. The cycle of development of *I. pileatus* is carried out with the participation of two intermediate hosts: freshwater snail of genus *Valvata* and fish species of the families: Cyprinidae, Percidae, Cobitidae, Gobiidae, Esocidae, Siluridae etc. The fish are intermediate hosts for parasite metacercaria, which grows in the body cavity under the serious cover of digestive organs and in the walls of the vesicle. The high abundance of the parasites causes massive extinction of fish hosts (Bauer, 1987; Kakacheva-Avramova, 1983; Sudarikov, 1984). *C. brachycollis* is intestinal parasite species of fish. The life cycle of *C. brachycollis* Janiszewska, 1951 is accomplished with the intermediate hosts *Limnodrilus hoffmeisteri* and *Tubifex tubifex*. Definitive hosts are fish species belonging to family Cyprinidae. Typical definitive hosts are fish species: *Barbus barbus*, *B. petenyi*, *Sq. cephalus*, *Leuciscus idus* (Kakacheva-Avramova, 1983; Bauer, 1987; Protasova, 1990; Scholz and Hanzelová, 1998; Barčák et al., 2017). Definitive hosts of *R. denudata* are a lot of fish species from family Cyprinidae. *R. denudata* is intestinal parasite species of fish. Intermediate hosts are invertebrates of genera Heptagenia, Ephemerella and of Hydropsyche

(Kakacheva-Avramova, 1983; Bauer, 1987; Moravec, 2013). *C. brachycollis* and *R. denudata* are authogenic parasites. They are species which life cycle is completing within the same freshwater ecosystem. The third species, *I. pileatus* is allogenic species, which use chub and another freshwater fish as intermediate hosts and mature in fish-eating birds – seagulls. *I. pileatus*, *C. brachycollis* and *R. denudata* are generalist, parasitizing in more than one different fish hosts.

Component communities

Helminths are fixed in 9 of 20 examined chub (45%). For each endoparasite species, prevalence (P%) and mean intensity (MI) are determined (Table 1). *R. denudata* is a core species (P%=30) of endoparasite communities of *Sq. cephalus* of the Osam River. *I. pileatus* is a component species (P%=10) and *C. brachycollis* is an accidental species (P%=5). With highest mean intensity was represented *I. pileatus* (MI=5), followed by *R. denudata* (MI=2.8). Only one specimen of *C. brachycollis* was established in one infected specimen of fish hosts (Table 1).

Table 1. Species diversity, prevalence (P%) and mean intensity (MI) of the endoparasites of *Squalius cephalus* from the Osam River

Species of endoparasites	Ecological indices (N[1]= 20)			
	n[2]	p[3]	P%[4]	MI[5]
Class Trematoda Order Strigeidida; Family Strigeidae				
Ichtyocotylurus pileatus (Rudolphi, 1802) Dubois, 1937 (metacercaria)	2	10 4-6	10	5
Class Cestoda Order Caryophyllaeidea; Family Caryophyllaeidae				
Caryophyllaeus brachycollis Janiszewska, 1951	1	1	5	1
Class Nematoda Order Spirurida; Family Rhabdochonidae				
Rhabdochona denudata (Dujardin, 1845) Raillet, 1916	6	17 2-6	30	2.8

[1]N = total number of examined fish specimens.
[2]n = total number of infected fish specimens.
[3]p = total number of endoparasite specimens.
[4]P% = prevalence.
[5]MI = mean intensity.

Infracommunities

A total of 11 examined specimens of chub are free of parasites (55%). No mixed infection has been established. Maximal numbers of endoparasites of examined specimens of chub are fixed for *I. pileatus* and *R. denudata* (on 6 specimens). The average number of endoparasite specimens, found in the total number of studied fish specimens is low (1.4±1.98). The value of Brillouin's diversity index isHB=0.689 (Table 2).

Table 2. Infracomminities data

No. of helminth species		
Number of fish	11	9
Number of helminth species	0	1
Number of helminth specimens		
Total number	28	
Mean±SD	1.4±1.98	
Range	2-6	
Mean HB±SD	0.611±0.145	

To this time, the endoparasites of *Sq. cephalus* in Bulgaria are presented with 28 species, belonging to the classes Trematoda, Cestoda, Acanthocephala and Nematoda (Table 3).

Table 3. Species of endoparasites of *Sq. cephalus* in Bulgaria

Species of endoparasites	**Authors**
Trematoda	
Allocreadium isoporum macrorchis	[4,5]
Allocreadium isoporumdubium	[10]
Pseudochetosoma salmonicola	[4,11]
Posthodiplostomum cuticola	[10,11]
Tylodelphys clavata	[5]
Nicola skrjabini	[8]
Apophallus mühlingi	[7]
Metagonimus yokogawai	[7,11]
Sphaerostomum bramae	[10]
Cestoda	
Caryophyllaeus laticeps	[10]
Caryophyllaeides fennica	[1,3,6]
Ligula intestinalis	[2,6,10]
Proteocephalus torulosus	[4,6,7,10]
Caryophyllaeus brachycollis Janiszewska, 1951	[4,6,10]
Caryophyllaeides fennica	[4,5]
Cestoidea gen sp	[6]
Shulmanella petruschewskii	[10]
Acanthocephala	
Acanthocephalus lucii	[1,7,10]
Acanthocephalus anguillae	[4,6,10]
Paracanthocephalus tenuirostris	[6]
Pomphorhynchus laevis	[1,7,9,10,11]
Neoechinorhynchus rutile	[10]
Nematoda	
Rhabdochona denudata (Dujardin, 1845) Raillet, 1916	[4,5,6,10,11]
Philometra abdominalis	[3,11]
Philometra ovate	[10]
Phylometra sp.	[4]
Rhaphydascaris acus	[10]
Cuculanus dogieli	[10]

[1]Margaritov, 1959.
[2]Bajlyozov et al., 1964.
[3]Margaritov 1964.
[4]Kakcheva-Avramova, 1969.
[5]Margaritov, 1977.
[6]Kakcheva-Avramova&Menkova, 1978.
[7]Kakcheva-Avramova et al., 1978.
[8]Nedeva, 1991.
[9]Nedeva et al., 2003.
[10]Cakis et al., 2004.
[11]Atanasov, 2012.

They are determined as a result of scientific survey of 11 authors in Bulgaria (Table 3).

These species are established mainly from the chub of the Danube River and its tributaries as well as of some lentic ecosystems of the Danube Basin in Northern Bulgaria.

The species *R. denudata* and *P. laevis*, followed by *P. torulosus,* and by *C. brachycollis, C. fennica* and *L. intestinalis* etc. are most frequently reported.

The three endoparasite species, found in the present study of the Osam River, represent only 10.71% of the established species for the country. *I. pileatus* is a new endoparasite species of the chub of the Osam River. *Sq. cephalus* is a new host record for *I. pileatus* in Bulgaria.

The species *C. brachycollis* and *R. denudata* are established of chub and of another fish species (Table 4), but they are reported for the first time of the freshwater ecosystem of the Osam River.

The knowledge of the life cycle of the established endoparasite species is testifying for the following paths for the circulating of the parasitic flow: A. Trematoda: 1. molluscs (*Valvata*) – fish – birds (*I. pileatus,* metacercaria); B. Cestoda: 1. Oligochaeta – fish (*C. brachycollis*); C. Nematoda: 1. larvae'sof Ephemeroptera and Diptera – fish (*R. denudata*).

Probably, the specified groups of intermediate hosts are represented with higher intensities of populations and are dominant species in the food ration of the chub of Osam River.

Tieri et al. (2006) examined metazoan parasites of *Sq. cephalus* of two rivers in Italy.

They reported 7 species of endohelminths, including *C. brachyollis* and *R. denudata*.

The prevalence and mean intensity of *C. brashycollis* showed higher values from both Italian rivers than these from the Osam River. In opposite, the prevalence and mean intensity of *R. denudata* from the Orta River were fixed with higher values than these from the Osam River, but they are more than two times lower of the Pescara River (more polluted), etc.

In general, the species diversity and characteristics of parasite communities of chub of the Osam River are low.

Table 4. Other species of fish in Bulgaria – hosts of the endoparasites found in *Sq. cephalus* from the Osam River

Species of endoparasites Fish species	***I. pileatus***	***C. brachycollis***	***Rh. denudata***
Alburnus alburnus (Linnaeus, 1758)	[13,17]	[4,21]	[4,5,8,13,17,21]
Leuciscus aspius (Linnaeus, 1758)			[3,27]
Barbus barbus (Linnaeus, 1758)		[5,10]	[1,5,29]
Barbus cyclolepis Heckel, 1837		[3,4,18,22]	[3,4,22]
Barbus petenyi Heckel, 1852		[5, 9,10,14]	[1,5]
Blicca bjoerkna (Linnaeus, 1758)	[8]		
Cobitis taenia Linnaeus, 1758			[10]
Cyprinus carpio Linnaeus, 1758	[6,7,11]		[15]
Gobio gobio (Linnaeus, 1758)			[5]
Leuciscus idus (Linnaeus, 1758)	[8]		
Rutilus rutilus (Linnaeus, 1758)		[4]	
Sander lucioperca (Linnaeus, 1758)	[8,12]		
Scardinius erythropthalmus (Linnaeus, 1758)	[8]		[2,26,27,29]
Squalius orpheus Kottelat & Economidis, 2006	[14,17,19,21,23, 30]	[3,4,5, 9,10, 14,17,19, 21,23,25,30]	[3,4,10, 14,16,17,19, 20,21,23,25,30]
Perca fluviatilis Linnaeus, 1758	[12,27,28]	[12,24]	
Vimba melanops (Heckel, 1837)		[4]	[4]
Zingel streber (Siebold, 1863)			[8]
Zingel zingel (Linnaeus, 1766)			[8]

[1]Margaritov, 1959.
[2]Kakacheva-Avramova, 1962.
[3]Kakacheva-Avramova, 1965.
[4]Margaritov, 1965.
[5]Kakacheva-Avramova, 1969.
[6]Margaritov, 1975.
[7]Margaritov, 1976.
[8]Kakacheva-Avramovaetal., 1978.
[9]Kakacheva-Avramova&Menkova, 1978.
[10]Kakacheva-Avramova&Menkova, 1981.
[11]Margaritov, 1992.
[12]Nedeva&Grupcheva, 1996.
[13]Kirin, 2001.
[14]Kirin, 2001a.
[15]Kirin, 2001b.
[16]Kirin, 2001c.
[17]Kirin, 2001d.
[18]Kirin2002.
[19]Kirin2002a.
[20]Kirin, 2002b.
[21]Kirinetal., 2002.
[22]Kirin, 2003.
[23]Kirin, etal., 2003.
[24]Cakisetal., 2004.
[25]Kirinetal., 2005.
[26]Shukerova&Kirin, 2008.
[27]Shukerova, 2010.
[28]Sukerovaetal., 2010.
[29]Atanasov, 2012.
[30]Kirinetal., 2013; etc.

CONCLUSIONS

River Osam is a new locality of *I. pileatus*, *C. brachycolli* and *R. denudata* in Bulgaria. *Sq. cephalus* is a new host record for *I. pileatus* in Bulgaria and Bulgarian part of the Danube Basin. *R. denudata* is a core species of the helminth communities.

The determined three endoparasite species represent only 10.71% of the established intestinal species of the chub in the country.

The low characteristic of infection indicated low biodiversity of the studied habitats and showed negative impacts on the areas of the examined freshwater ecosystem.

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