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NEW DATA FOR HELMINTH COMMUNITIES OF *Alburnus alburnus* (Linnaeus, 1758) FROM MARITSA RIVER, BULGARIA

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Abstract

During summer 2018, 29 specimens of bleak (Alburnus alburnus (Linnaeus, 1758)) from Maritsa River were collected and examined with standard techniques for parasites. Helminth parasites were recorded in 24 bleak specimens (82.75%) from Maritsa River. Five species of parasites were identified: one trematode species (Allocreadium isoporum (Looss, 1984)), two cestode species (Caryophyllaeus brachycollis Janiszewska, 1951, Ligula intestinalis (Linnaeus, 1758)); *one acantocephalan species (Pomphorhynchus laevis (Müller, 1776)) and one nematode species (Rhabdochona denudata (Dujardin, 1845; Raillet, 1916). The analysis of the dominant structure of the found parasite species is presented to the component and infracommunities level. In the component community of Alburnus alburnus from Maritsa River C. brachycollis, P. laevis and, R. denudata are corespecies. L. intestinalisis component parasite species and A. isoporum is accidental parasite species for the helminth communities of bleak. The infracommunities data was used to be fixed principal biotic indices. Bioindicator significance of established parasite species was discussed for ecological evaluation of the state of the studied freshwater ecosystem.New data for the helminths and helminth communities of bleak from Maritsa River are presented.*

Key words*: Alburnus alburnus; bioindication; helminth communities; Maritsa River.*

INTRODUCTION

The Maritsa River (Maritsa/Meriç/Evros) is the longest river (539 km, of which 321.6 km on Bulgarian territory) that runs solely in the interior of the Balkan Peninsula. The Maritsa River springs from the Rila Mountains (2378 m altitude) in Western Bulgaria, flowing southeast between the Balkan and Rhodope Mountains and passes through the districts of Pazardzhik, Plovdiv, Stara Zagora and Haskovo. After leaving Bulgaria, the river passes through the north-eastern part of Greece and the European part of Turkey and enters the Aegean Sea. The Maritsa is the fourth longest river in Bulgaria and is included in the National monitoring program (Regulation 1/2011).

Fish parasite communities and biodiversity from the Maritsa River and the state of the freshwater ecosystem, including metal content in the fish host and parasites were studied (Margaritov, 1965; Kirin, 2000a; 2000b; 2001a; 2006; 2013; 2014), but only Margaritov (1965) examined specifically bleak. The aim of this study is to present the diversity and communities of endoparasites of bleak (*Alburnus alburnus* (Linnaeus, 1758)) from Maritsa River, Bulgaria. As a result of this survey new data for helminths and helminth communities of *A. alburnus* is presented.

MATERIALS AND METHODS

During the summer of 2018, fish and fish parasites were collected and examined from the Maritsa River (in the vicinityof the city of Plovdiv). The city of Plovdiv (42°9′N 24°45′E) is situated on the two banks of the Maritsa River. The region of the city and the riverside are distinguished with a significant diversity of highly protected species and territories declared as protected with national and international nature protective status (Assyov, 2012).

A total of 29 bleak specimens (*Alburnus alburnus* (Linnaeus, 1758)) were collected and examined from the Maritsa River during the summer of 2018. The fish were caught by angling. The scientific and common names of the fish host are used according to the FishBase database (Fröse and Pauly, 2018). The fish

were immediately after their capture examined for gastrointestinal and tissue helminths (an incomplete parasitological study). Helminths were cleaned in a saline solution and fixed in 70% ethanol. Trematodes were fixed as permanent slides after their colouring with acetic carmine, differentiation in 70% acid ethanol, dehydrating in increasing ethanol series, clarifying in eugenol and mounting in Canada balsam (Bykhovskaya-Pavlovskaya, 1985; Georgiev et al., 1986). The samples were counted and identified using keys of Bauer et al. (1981) and Bykhovskaya-Pavlovskaya (1985). Cestodes were stained with acetic carmine and mounted as permanent slides in a Canada balsam according to Georgiev et al. (1986) and Scholz and Hanzelová (1998). Acanthocephalans were examined as temporary slides in ethanol-glycerin and identified (Petrochenko, 1956; Ergens and Lom, 1970; Bykhovskaya-Pavlovskaya, 1985). Nematodes were examined as temporary microscopic preparations in glycerin (Moravec, 1994; 2013).

The dominant structure of the component helminth communities was determined according to the criteria proposed by Kennedy (1993) on the basis of the prevalence (P%): accidental (P%<10), component (10<P%<20) and core (P%>20) species. The ecological terms prevalence, mean intensity are used, based on the terminology of Bush et al. (1997). Analyses of helminth community structure were carried out in both levels: infracommunity and component community. The component data were used to determine the total number of species, Shannon diversity index (H'), Pielou evenness index (E), Berger-Parker dominance index (d) according to Magurran (2004). The infracommunity data was used to calculate the mean number of species, the mean number of helminth specimens, Brillouin diversity index (HB) (Kennedy, 1993; 1997; Magurran, 2004).

RESULTS AND DISCUSSIONS

A total of 29 specimens of bleak (*Alburnus alburnus* (Linnaeus, 1758)) were collected and examined from the Maritsa River. *Alburnus alburnus* is estimated as least concern species (LC=Least Concern; IUCN Red List Status). Bleak is freshwater, brackish, benthopelagic, potamodromous fish species. This fish species inhabit open waters of lakes and medium to large rivers. Larvae of *A. alburnus* live in the littoral zone of rivers and lakes, while juveniles leave shores and occupy a pelagic habitat, feeding on plankton, drifting insects or invertebrates fallen on the water surface. The diet of this fish includes mainly plankton, as well as crustaceans and insects (Fröse and Pauly, 2018).

Helminth parasites were recorded in 24 bleak specimens (85.75%) from the Maritsa River. Five species of parasites were identified: one trematode species (*Allocreadium isoporum* (Looss, 1984)), two cestode species (*Caryophyllaeus brachycollis* Janiszewska, 1951), *Ligula intestinalis* (Linnaeus, 1758)); one acantocephalan (*Pomphorhynchus laevis* (Müller, 1776)) and one nematode species (*Rhabdochona denudata* (Dujardin, 1845) Raillet, 1916) (Table 1).

All helminth species occurred as adults with the exception of *L. intestinalis*. *A. isoporum, C. brachycollis, P. laevis* and *R. denudate* are autogenic species, matured in fish. *L. intestinalis* is allogenic species. The larval stages of *L. intestinalis* develop in the body cavity of carp fishes – *Abramis brama, A. sapa, S. erythrophtalmus, A. alburnus, A. bipunctatus, Gobio gobio, Rutilus rutilus, Barbus barbus, B. m. petenyi, Leuciscus cephalus, L. idus* and *Phoxinus phoxinus* (Kakacheva-Avramova, 1983). In an adult state *L. intestinalis* parasitized in fish-eating birds, mainly gulls (*Larus*), less commonly in fish-eating ducks (*Bucephala* and *Mergus*) and in *Podiceps* (Kakacheva-Avramova, 1983).

In the component community of *Alburnus alburnus* from the Maritsa River *C. brachycollis* (P%=24.13), *P. laevis* (P%=31.03) and *R. denudata* (P%=37.93) are core species. *L. intestinalis* (P%=10.34) is component parasite species and *A. isoporum* (P%=6.89) is accidental parasite species for the helminth communities of bleak (Table 1).

Table 1. Helminth parasites of *Alburnus alburnus* from Maritsa River (N – number of examined hosts, n – number of infected hosts, p – number of parasites, P – prevalence, MI – mean intensity, MA – mean abundance)

Helminth species	N=29					
	N	P	P%	MI±SD	MA±SD	Range
Allocreadium isoporum (Looss, 1984)	2	3	6.89	1.5±0.5	0.10±0.40	1-2
Caryophyllaeus brachycollis (Janiszewska, 1951)	7	11	24.13	1.57±0.49	0.37±0.71	1-2
Ligula intestinalis (Linnaeus, 1758)	3	3	10.34	1.0±0	0.10±0.30	1
Pomphorhynchus laevis (Müller, 1776)	9	16	31.03	1.77±0.78	0.55±0.93	1-3
Rhabdochona denudata (Dujardin, 1845) Raillet, 1916	11	18	37.93	1.63±0.48	0.62±0.84	1-2

In the component community of *Alburnus alburnus* from the Maritsa River nematodes are presented with the highest number of specimens, with 1 species and 18 specimens. Acanthocephalans are presented with one species and 16 specimens. Cestodes are represented by two species and 14 specimens. Trematodes are represented by one species and 3 specimens.

Allocreadium isoporum was found in *A. alburnus* from Danube River (Kakacheva-Avramova, 1977). For Maritsa River, *A. isoporum* was reported as parasite of *Squalius cephalus* (Linnaeus, 1758) (*Leuciscus cephalus*) and *Barbus cyclolepis* (Heckel, 1837) (*Barbus tauricus cyclolepis*) (Margaritov, 1965). For Maritsa River and Stryama River was also reported the subspecies *Allocreadium isoporum macrorchis* with host *Sq. cephalus* (*L. cephalus*) (Kirin, 2000a; 2000b; 2001a; Kirin et al., 2002). *A. isoporum* is an intestinal parasite of many species of family Cyprinidae (Kakacheva-Avramova, 1983). *A. isoporum* develops with the participation of two intermediate hosts: the first are bivalves of the genus *Sphaerium*, and the second is an insect larva of the genus *Ephemera*, *Anabolia* and *Chaetopteryx* (Kakacheva-Avramova, 1983).

Representatives of the genus *Sphaerium* and genus *Anabolia* are bioindicators for α-mesosaprobity and β-mesosaprobity (Johnson et al., 1993). Representatives of the genus *Ephemera* are bioindicators for β-mesosaprobity and oligosaprobity (Johnson et al., 1993). Representatives of the genus *Chaetopteryx* are bioindicators for β-mesosaprobity (Johnson et al., 1993).

Caryophyllaeus brachycollis was found in *A. alburnus* from Maritsa River, Danube River and Arda River (Margaritov, 1965; Kakacheva-Avramova, 1977; Kirin et al., 2002). For Maritsa River *C. brachycollis* was reported as parasite of *Sq. cephalus* (*L. cephalus*), *B. cyclolepis* (*B. tauricus cyclolepis*), *Vimba melanops* and *Rutilus rutilus* (Margaritov, 1965; Kirin, 2000a; 2001a; 2014). Intermediate host of *C. brachycollis* is *Limnodrilus hoffmeisteri* and definitive hosts are freshwater fish species from family Cyprinidae (Kulakovskaya, 1961; Kakacheva-Avramova, 1983).

Limnodrilus hoffmeisteri is bioindicator for polisaprobity (Johnson et al., 1993).

Ligula intestinalis was reported as parasite of *Alburnus alburnus* from Palakariya River, Arda River and Danube River (Kakacheva-Avramova and Nedeva-Menkova, 1978; Kirin et al., 2002; Chunchukova et al., 2018).

Ligula intestinalis (Diphyllobothriidae) is widely distributed cestode species with a complex life cycle, which involves a copepod as the first intermediate host, fish as a second intermediate host and an avian definitive host (Dubinina, 1980).

P. laevis was reported as a parasite of *A. alburnus* from Struma River and Danube River (Kakacheva-Avramova, 1962; 1977; Margaritov, 1966; Atanasov, 2012; Chunchukova et al., 2018). For Maritsa River *P. laevis* was reported as parasite of *Sq. cephalus* (*L. cephalus*) and *Esox lucius* (Kirin, 2000a; 2000b; 2001a; 2006).

Intermediate host of *P. laevis* is *Gamma ruspulex*, definitive hosts are fish most often from family Cyprinidae and paratenic hosts are small fish of the same family (Kakacheva-Avramova, 1983). *G. pulex* is a bioindicator for β-mesosaprobity (Johnson et al., 1993).

R. denudate was reported as a parasite of *A. alburnus* for the rivers Struma, Tundja, Maritsa, Arda, Veleka, Resovka and Danube (Kakacheva-Avramova, 1962; 1970; 1977; Margaritov, 1965; Kirin, 2001b; 2003; Kirin et al., 2002). For Maritsa River, *R. denudate* was reported as a parasite of *Sq. cephalus* (*L. cephalus*), *Vimba melanops* (Heckel, 1837) (*Vimba vimba melanops*), *Alburnus alburnus* and *Barbus cyclolepis* (*B. tauricus cyclolepis*) (Margaritov, 1965; Kirin, 2000; 2001b).

Rhabdochona denudata is an intestinal parasite of many species of family Cyprinidae (Moravec, 2013). Intermediate hosts of *R. denudata* are insect larvae: *Heptagenia* sp., *Ephemerella* sp. and *Hydropsyche* sp. (Bauer, 1987; Kakacheva-Avramova, 1983). Moravec (2013) suggested that in addition to mayflies also some other aquatic arthropods may serve as intermediate hosts of *R. denudata.*

Representatives of the genera *Heptagenia* and *Ephemerella* are bioindicators for β-mesosaprobity. *Hydropsyche* sp. is bioindicator for 0-α-mesosaprobity (Johnson et al., 1993). Species richness in infracommunityof bleak ranges from 0 to 3 species. With 1 helminth species were infected 17 specimens of fish (58.62%): 6 bleaks (20.69%) with *R. denudata*; 6 bleaks (20.69%) with *P. laevis*; 4 bleaks (13.79%) with *C. brachycollis* and only one bleak (3.44%) with *L. intestinalis*. With 2 helminth species were infected 6 specimens of *A. alburnus* (20.69%). Only one specimen of bleak (3.44%) was infected with 3 helminth species (*A. isoporum*, *C. brachycollis* and *R. denudata*). Five of the studied bleak specimens were free of parasites (17.24 %).

The largest number of helminth specimens established in a single host specimen is 5. The average species richness (mean number of species for a fish specimen) in infracommunity of bleak is 1.10±0.71 species (Table 2).

Average abundance (mean number of helminths in fish) in these infracommunities is 1.76±1.36. The parasite communities of *A. alburnus* from the Maritsa River showed Brillouin diversity index, HB=1.26 (Table 2).

Table 2. Infracommunities of *Alburnus alburnus* from Maritsa River

Alburnus alburnus	Number of endohelminth species					
	0	1	2	3	Mean±SD	Range
	5	17	6	1	1.10±0.71	0-3
Alburnus alburnus	Number of endohelminth specimens					
	Total number		Mean±SD		Range	Brillouin's index HB
	51		1.76±1.36		0-5	1.26

In general, the parasite communities of *A. alburnus* are represented by 5 species of parasites belonging to four classes, five orders, and five families. The total number of isolated and studied helminth specimens was 51. The obtained results were related to a Brillouin diversity index HB=1.26, Shannon diversity index H'=1.395, Berger-Parker dominance index d=0.353 and Pielou evenness index E=0.867.

The circulation of parasitic flow in the studied freshwater ecosystem can be represented as follows: class Cestoda: aquatic worms - fish (*Caryophyllaeus brachycollis*) and crustaceans - fish - birds (*Ligula intestinalis*); for class Acanthocephala: crustaceans - fish - fish

(*Pomphorhynchus laevis*); Class Nematoda: insect larvae - fish (*Rhabdochona denudata*).
During this study of helminth communities of *A. alburnus* five parasite species were found. In the only previous research of helminth fauna of bleak from Maritsa River, Margaritov (1965) reported three species (Table 3). In this study, the highest prevalence was detected for *Rhabdochona denudate* (P%=37.93). Margaritov (1965) also reported *R. denudata* as the species with the highest prevalence in helminth communities of bleak, but with higher values (P%=55). This study of endohelminth community of *A. alburnus* from the Maritsa River corresponds only partially with previous survey by Margaritov (1965) (Table 3).

Table 3.Overview of helminth species of *Alburnus alburnus* registered in the Maritsa River

Authority / Helminth species	Margaritov (1965)	This study
Class Trematoda		
Allocreadium isoporum		●
Class Cestoda		
Caryophyllaeus brachycollis	●	●
Ligula intestinalis		●
Class Acanthocephala		
Acanthocephalus anguillae	●	
Pomphorhynchus laevis		●
Class Nematoda		
Rhabdochona denudata	●	●

CONCLUSIONS

This study presents new data forthe bleak's (*Alburnus alburnus* (Linnaeus, 1758)) endohelminth species and structure of helminth communities from Maritsa River, Bulgaria. Helminth parasites were recorded in 24of the examined bleak specimens (85.75%) from the Maritsa River.Five species of parasites were identified: one trematode species (*Allocreadium isoporum* (Looss, 1984)), two cestode species (*Caryophyllaeus brachycollis* Janiszewska, 1951, *Ligula intestinalis* (Linnaeus, 1758)); one acantocephalan (*Pomphorhynchus laevis* (Müller, 1776)) and one nematode species (*Rhabdochona denudata* (Dujardin, 1845) Raillet, 1916).
The established in this study *A. isoporum, L. intestinalis* and *P. laevis* are reported for the first time for *A.alburnus* from Maritsa River.
The obtained results for the parasite communities are related to Brillouin diversity index HB=1.26 and Pielou evenness index E=0.867.
The obtained results for the parasite communities and the bioindicative role of their intermediate hosts are demonstrating a favourable development of the studied freshwater ecosystem.

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