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STUDIES ON THE HELMINTH FAUNA OF TWO FISH SPECIES OF THE GENUS *Ballerus* Heckel, 1843 FROM THE BULGARIAN SECTION OF THE DANUBE RIVER

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Abstract

During the period 2019-2021, the helminth fauna of two species of freshwater fish of the family Cyprinidae, genus Ballerus Heckel, 1843 - white-eye bream (Ballerus sapa (Pallas, 1814)) and zope (Ballerus ballerus (Linnaeus, 1758)) were examined. Six specimens of white-eye bream and one specimen of zope were collected from a total of three biotopes located in the Bulgarian section of the Danube River between 845 and 807 river km. Three species of helminths were found - 2 species of the class Trematoda (Asymphylodora imitans (Mühling, 1898) Looss, 1899; Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960)) and 1 species of the class Nematoda (Contracaecum sp. (larvae)). The present study aims to provide new data on the species composition and helminth ecological indices of the two examined fish species. B. sapa is reported as a new host record for the three helminth species in Bulgaria. Koshava biotope is a new habitat for the established helminths in the white-eye bream.

Key words*: Ballerus ballerus, Ballerus sapa, ecological indices, helminths, Vidin Province.*

INTRODUCTION

The Danube River, with a length of 2,850 km, ranks among the longest rivers in Europe. The length of the Danube River in Bulgarian territory is 470 km. The Bulgarian section of the river starts from the mouth of the Timok River at the Danube River (at 845 river km) and reaches the town of Silistra (at 375 river km). The river is distinguished by exceptional biological diversity, with 68 fish species reported for the Bulgarian section (Zarev et al., 2013). Species from the families Cyprinidae, Percidae, Gobiidae, Cobitidae, and others predominate (Keckeis & Schiemer, 2002). Different authors study the species composition of parasites of white-eye bream from the Danube River in Bulgaria (Kakacheva-Avramova et al., 1978; Kirin et al., 2013); in Serbia (Đikanović et al., 2013). There are also studies from the river basin in Romania (Cojocaru, 2003); Slovakia (Oros & Hanzelová, 2009; Hanzelová et al., 2011), and Serbia (Djikanovic et al., 2011). Studies on the parasite fauna of zope have been conducted from the Bulgarian section (Kakacheva-Avramova et al., 1978) and Serbian section (Đikanović et al., 2013) of the Danube River, as well as for the river basin in Romania (Cojocaru, 2003) and Serbia (Djikanovic et al., 2011).

The present study aims to study the species composition of the helminths of two fish species of genus *Ballerus* Heckel, 1843, inhabiting the freshwater ecosystem of the Danube River in Bulgaria; to provide new data on the mean intensity, mean abundance, and prevalence of endohelminths of the studied fish species.

MATERIALS AND METHODS

The objects of study are white-eye bream (*Ballerus sapa* (Pallas, 1814)) (syn. *Abramis sapa* (Pallas, 1814)) and zope (*Ballerus ballerus* (Linnaeus, 1758) (syn. *Abramis ballerus* (Linnaeus, 1758)). The fish were caught from the Danube River, on the vicinities of three villages - Kudelin, Novo selo, and Koshava (designated as biotopes), located in the border zone of Bulgaria, Vidin Province. The biotopes are located at 844, 833 and 807 km along the Danube River, respectively (Figure 1).

Fish were caught according to BS EN 14757:2015 Water quality - Sampling of fish

with multi-mesh gillnets. Net fishing devices were used under permits issued by the Executive Agency for Fisheries and Aquaculture. Scientific species names were given according to Fröse & Pauly (2022). Immediately after capture, all fish specimens were measured and weighed. Mean values for total body length (TL), maximum body height (MH), and body weight (BW) of *B. sapa* specimens were calculated: 16.12 ± 6.39 cm, 4.33 ± 1.79 cm and 49.17 ± 39.71 g, respectively.

Figure 1. Location of the studied section of the Danube River (844-807 river km), Vidin Province, Bulgaria (https://www.google.bg/maps/place/Видин)

The captured specimens white-eye bream and zope were subjected to helminthological examination by methods indicated by Zashev & Margaritov (1966); Kakacheva-Avramova (1983); Bauer (Ed.) (1987); Moravec (2013); others. For all types of helminths ecological indices were calculated: mean intensity (MI); mean abundance (MA) and prevalence (P%) according to Bush et al. (1997). The helminthological studies were carried out in the laboratory of the Department of Agroecology and Environmental Protection, Agricultural University - Plovdiv. To determine the taxonomic affiliation of the isolated parasites, a microscope "XS-213", China, was used.

RESULTS AND DISCUSSIONS

Fish species

Two fish species of the genus *Ballerus* Heckel, 1843; family Cyprinidae were selected as model fish species. *B. sapa* is a freshwater, brackish and benthopelagic fish. Inhabits fast-flowing waters. The diet of the species consists of a variety of crustaceans, molluscs, and aquatic vegetation. *B. ballerus* is a freshwater, benthopelagic fish. It occurs in slow-flowing waters. Uses zooplankton for food. Both species grow at a slow rate. The body length of white-eye bream and zope is up to 30 cm and 45 cm, respectively, and the weight is up to 800 g and up to 1.5 kg, respectively. Visually, the two species are similar, as zope is distinguished from white-eye bream by the position of the mouth, which is upturned; by the snout, which is pointed; by the smaller scales; and by the size of the eyes, which are smaller (Karapetkova & Zhivkov, 2006; Kottelat & Freyhof, 2007). They are included in the IUCN Red List with the category "Least Concern" and Annex III of the Bern Convention (Convention on the conservation of European wildlife and natural habitats, 1982; Freyhof & Brooks, 2011).

Ecologohelminthological examinations

A total of six specimens *B. sapa* (5 specimens from Koshava biotope and 1 specimen from Novo selo biotope) and one specimen *B. ballerus* from the Kudelin biotope were subjected to helminthological examination. Infection was found only in white-eye bream from Koshava biotope. Two out of five examined specimens of *B. sapa* (40%) were infected. Three types of endohelminths - two species of class Trematoda (*Asymphylodora imitans* (Mühling, 1898) Looss, 1899; *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960) and one species of class Nematoda (*Contracaecum* sp. (larvae)) were found (Table 1).

Table 1. Taxonomic position, synonyms, localization, biotopes, season of detection of *Asymphylodora imitans*, *Nicolla skrjabini*, and *Contracaecum* sp.

Helminth species	*Asymphylodora imitans*[1]	*Nicolla skrjabini*[2,3]	*Contracaecum* sp.
Taxonomic position	CLASS TREMATODA RUDOLPHI, 1808 Family Monorchidae Odhner, 1911 Genus *Asymphylodora* Looss, 1899	CLASS TREMATODA RUDOLPHI, 1808 Family Opecoelidae Ozaki, 1925 Genus *Nicolla* Wišniewski, 1933	CLASS NEMATODA RUDOLPHI, 1808 Family Anisakidae Skrjabin et Karokhin, 1945 Genus *Contracaecum* Railliet & Henry, 1912
Synonyms[1,2]	*Asymphylodora dneproviana* Iwanitzky, 1928; *Distoma imitans* Mühling, 1898	*Coitocaecum macrostomum* Pigulewsky, 1931; *Coitocaecum ovatum* Pigulewsky, 1931; *Coitocaecum skrjabini* Iwanitzky, 1928; *Coitocaecum macrostomum* Pigulewski, 1931; *Coitocaecum ovata* Pigulewski, 1931; *Crowcrocaecum skrjabini* (Iwanitzky, 1928) Skrjabin & Koval, 1956; *Excoitocaecum skrjabini* (Ivanitzkii, 1928) Slusarski, 1958; *Nicolla macrostoma* (Pigulewsky, 1931) Wisniewski, 1934; *Nicolla ovata* (Pigulewsky, 1931) Wisniewski, 1934; *Nicollia macrostoma* (Pigulewsky, 1931) Wisniewski, 1933; *Nicollia ovata* (Pigulewsky, 1931) Wisniewski, 1933	-
Localization	Intestine	intestine	in capsules on the serous membrane of the organs in the abdominal cavity of the fish
Biotope	Koshava	Koshava	Koshava
Season	Spring	spring	spring

[1]WoRMS (2022a); [2]WoRMS (2022b)

In the present study, a total of 711 endohelminth specimens were isolated. The trematode *As. imitans* had the highest values for MI and MA (MI = 700.00 and MA = 140.00), and the nematode *Contracaecum* sp. had the lowest (MI = 3.00 and MA = 0.60). All three isolated species of helminths had equal prevalence (P% = 20.00) (Table 2).

Table 2. Species diversity and ecological indices in the helminth community of *Ballerus sapa* from the Danube River

Ballerus sapa **(N = 5 / Koshava)** **Parasite species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Asymphylodora imitans (Mühling, 1898) Looss, 1899	1	700	700.00	140.00	20.00	700
Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960	1	8	8.00	1.60	20.00	8
Contracaecum sp. (larvae)	1	3	3.00	0.60	20.00	3

N - number of investigated fish; n - number of infected fish; p - number of fish parasites; MI - mean intensity; MA - mean abundance; P% - prevalence; R - range

Definitive hosts of *As. imitans* are freshwater fish species, such as *Blicca bjoerkna* (Linnaeus, 1758); *Abramis brama* (Linnaeus, 1758); *B. ballerus*; *B. sapa*; *Scardinius erythrophthalmus* (Linnaeus, 1758). The development cycle of this helminth species is not sufficiently studied. Definitive hosts of *N. skrjabini* are fish species of families Cyprinidae, Percidae, Gobiidae, Cobitidae, Siluridae, Gadidae, Esocidae, Acipenseridae, Salmonidae. The specific hosts are *Gymnocephalus acerina* (Gmelin, 1789) and *Silurus glanis* Linnaeus, 1758. *N. skrjabini* has a one-year development cycle involving two intermediate hosts. The first intermediate host is the snail *Lithoglyphus naticoides* (Pfeiffer,

1828). In it, sporocysts develop, localized in the liver, gonads, and gills. The second intermediate hosts are the crustaceans *Gammarus balcanicus* Schäferna, 1923; *Pontogammarus crassus* (Sars, 1894); *Dikerogammarus haemobaphes* (Eichwald, 1841). The metacercariae encyst in the dorsal musculature and fins. They are also found in the body cavity of crustaceans (Bykhovskaya-Pavlovskaya et al., 1962; Gaevskaya et al., 1975; Kakacheva-Avramova, 1983; Bauer (Ed.), 1987). *Contracaecum* sp. has definitive hosts of waterfowl (*Ardea*, *Egretta*, *Podiceps*, *Phalacrocorax*). Intermediate hosts are copepods (*Cyclops*, *Acanthocyclops*, *Macrocyclops*, *Mesocyclops*, *Eucyclops*, *Arctodiaptomus*, *Diaptomus*) (Bauer (Ed.), 1987; Moravec, 2013).

During a parasitological examination of *B. sapa* from the Bulgarian section of the Danube River *Apophallus muehlingi* (Jägerskiöld, 1899) Lühe, 1909; *Caryophyllaeus laticeps* (Pallas, 1781) Lühe, 1910; *Caryophyllaeides fennica* (Schneider, 1902) Nybelin, 1922; *Contracaecum bidentatum* (Linstow, 1899); *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911 and *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908 (Kakacheva-Avramova et al., 1978) were reported in the area of the cities of Ruse, Svishtov, Silistra, Lom, Tutrakan; and *Pomphorhynchus tereticollis* (Rudolphi, 1809) Meyer, 1932 (Kirin et al., 2013) in the area of the village of Vetren. For the parasite fauna of *B. sapa* from the Serbian section of the Danube River near Zemun (1,173 river km) and Visnjica (1,162 river km) *C. fennica*; *Caryophyllaeus* sp.; *C. laticeps*; *Triaenophorus nodulosus* (Pallas, 1781) Rudolphi, 1793; *Proteocephalus* sp.; *Proteocephalus torulosus* (Batsch, 1786) Nufer, 1905; *Ligula intestinalis* (Linnaeus, 1758) Gmelin, 1790; cestode cysts were reported (Đikanović et al., 2013). White-eye bream from the Danube River basin in Serbia (Djikanovic et al., 2011), in Slovakia – Tisa and Latorica rivers (Oros & Hanzelová, 2009; Hanzelová et al., 2011), and Romania – Timiş and Bega rivers (Cojocaru, 2003) was examined for the presence of parasites. Djikanovic et al. (2011) found *Rhipidocotyle campanula* (Dujardin, 1845); *Ap. muehlingi*; *C. fennica*; *Tr. nodulosus*; *Pr. torulosus*; *L. intestinalis*; *Acanthocephalus tenuirostris* (Achmerov & Dombrovskaja-Achmerova, 1941) Yamaguti, 1963. Oros & Hanzelová (2009) and Hanzelová et al. (2011) reported the helminths *C. fennica*; *Caryophyllaeus brachycollis* Janiszewska, 1953; *C. laticeps* (for Tisa River) and *Aspidogaster limacoides* Diesing, 1834; *As. imitans*; *Palaeorchis incognitus* Szidat, 1943; *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960 (syn. *Crowcracoecum skrjabini*); *Nicolla testiobliqua* (Wisniewski, 1933) Dollfus, 1958; *C. fennica*; *C. laticeps* (for Latorica River). Cojocaru (2003) found *As. imitans* (Table 3).

Table 3. Distribution of the found helminths (in the present study) of *Ballerus sapa* from the Danube River and its basin

Biotopes / Helminth species	Koshava biotope	Novo selo biotope	Danube River in other countries	Danube River Basin in other countries	Danube River in Bulgaria	Danube River Basin in Bulgaria
Asymphylodora imitans (Mühling, 1898) Looss, 1899	+	-	-	+	-	-
Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960	+	-	-	+	-	-
Contracaecum sp.	+	-	-	-	-	-

During a study of the parasite fauna of *B. ballerus* from the Bulgarian section of the Danube River in the area of Vidin, Silistra, Svishtov, Lom, Ruse, Tutrakan, the helminths *N. skrjabini*; *C. fennica* and *P. laevis* were found (Kakacheva-Avramova et al., 1978). For zope from the Serbian section of the river near Zemun (1,173 river km) and Visnjica (1,162 river km) the cestodes *C. fennica*; *C. laticeps*; *Proteocephalus* sp.; *Pr. torulosus*; cestode cysts were reported (Đikanović et al., 2013). Cojocaru (2003) studied *B. ballerus* from the Danube River basin in Romania (Timiş and Bega rivers) and reported the trematode *As.*

imitans. Djikanovic et al. (2011) reported *Pr. torulosus*; *Philometra ovata* (Zeder, 1803), and others of zope from the Danube River basin in Serbia.

CONCLUSIONS

During the period 2019-2021, five specimens of *B. sapa* from Koshava biotope; one specimen *B. sapa* from Novo selo biotope, and one specimen *B. ballerus* from Kudelin biotope were examined for helminths. Infection was found only in white-eye bream from Koshava biotope. Three taxa of helminths - *As. imitans* and *N. skrjabini* (class Trematoda), and *Contracaecum* sp. (class Nematoda), were found. *As. imitans* was distinguished by the highest mean intensity (MI = 700.00) and mean abundance (MA = 140.00). All three parasite species had an equal prevalence (P% = 20.00). Koshava biotope is a new habitat for the found helminths of white-eye bream. *B. sapa* is a new host for *As. imitans*, *N. skrjabini* and *Contracaecum* sp. in Bulgaria.

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