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Original Article

New data on the parasites and parasite communities of *Rutilus rutilus* (Linnaeus, 1758) from the Danube River, Bulgaria

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Abstract: The present study aims to provide new data on the parasites and the first data on the parasite communities of the roach *Rutilus rutilus* (Linnaeus, 1758) from the Bulgarian section of the Danube River. The fish were caught in 2019-2021 from three biotopes (Koshava, Kudelin, and Novo Selo). Fish samples were collected during spring, summer, and autumn. A total of 152 *R. rutilus* specimens were examined. Nine helminth species were isolated: three species of Trematoda, two Cestoda, two Acanthocephala, and two Nematoda. The component community and infracommunity of the roach from the Danube River, Kudelin biotope, were examined. *Rutilus rutilus* is a new host record for six helminth species in Bulgaria.

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Introduction

The Danube River passes through the territory of ten European countries. The river ranks second in length in Europe (Juhásová et al., 2019). The catchment area of the Danube River is the second largest in Europe, covering an area of 817,000 km² (Kotsev and Zhelezov, 2014). The Bulgarian section of the Danube River extends from 845 to 375 river km. Sixty-eight fish species have been reported for the Bulgarian section of the river (Zarev et al., 2013). Species of the family Cyprinidae are among the most common fish in Bulgarian water bodies (Zhelyazkov et al., 2018).

The parasites' life cycles occur with the hosts' participation (Hamood, 2021). Freshwater fish species serve as hosts for numerous parasite species (Docan et al., 2021). Studies on the parasite fauna of roach (*Rutilus rutilus*) in the Danube River are few. Parasitological studies on the species have been conducted in Romania (Stroe et al., 2021, 2022) and Bulgaria (Kakacheva-Avramova et al., 1978; Atanasov, 2012; Kirin et al., 2013). Research on parasites of roach caught from the river basin was conducted on the territory of Slovakia (Hanzelová et al., 2009; Oros and Hanzelová, 2009), Serbia (Djikanovic et al., 2011), Romania (Docan et al., 2021), Bulgaria (Shukerova, 2010; Shukerova and Kirin, 2019), and others. Therefore, this study aims to present the results of a parasitological investigation on *R. rutilus* from the Kudelin, Novo Selo, and Koshava biotopes, as well as new data on the parasite communities of the roach from the Kudelin biotope, specifically the Danube River.

Materials and Methods

For 2019-2021, a parasitological examination of 152 *R. rutilus* specimens was carried out. The fish samples were collected from the Danube River in the vicinity of three villages. The sampling sites are designated as biotopes – Kudelin biotope (44°12'07.9"N 22°41'28.2"E), Novo selo biotope (44°08'56.0"N 22°48'56.0"E), and Koshava biotope (44°03'59.9"N 23°02'10.2"E) (Fig. 1).

Fish samples were collected in accordance with BDS EN 14757:2015. The catch was made in three

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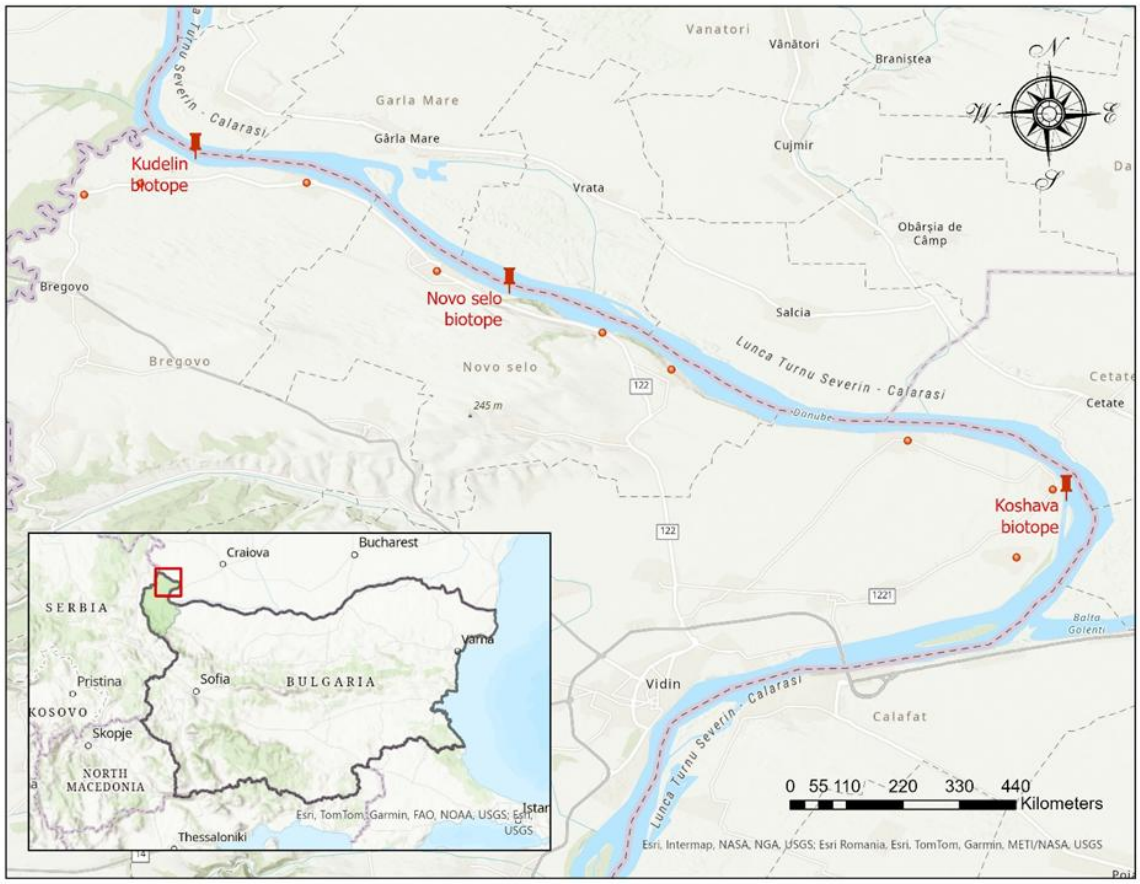

Figure 1. Location of the studied biotopes along the Danube River, Vidin Province, Northwestern Bulgaria

seasons (spring, summer, and autumn). The fish species was identified using proper references (Karapetkova and Zhivkov, 2006; Froese and Pauly, 2024). The roach, *Rutilus rutilus* (Linnaeus, 1758) (Cyprinidae), is a freshwater, brackish fish that inhabits water bodies rich in aquatic vegetation. It is an omnivorous species. The species' diet consists of crustaceans, insects, and aquatic vegetation. It is a widespread species in Bulgaria, found in the Danube River and its basin, as well as in some dams. The species is included in the IUCN Red List as "Least Concern" (Karapetkova and Zhivkov, 2006; Freyhof and Brooks, 2011).

Isolated acanthocephalans and nematodes were mounted on temporary slides with glycerin, while isolated trematodes and cestodes were mounted on permanent slides with Canada balsam (Dubinina, 1948; Scholz and Hanzelová, 1998; Moravec, 2013). A microscope (XS-213) was used to determine the taxonomic affiliation of the parasites. Parasite communities of the roach were represented at two levels – component community and infracommunity. Species in the component community of the roach were divided into core (P% > 20), component (10 < P% < 20), and accidental species (P% < 10) based on their prevalence (Kennedy, 1993). The ecological indices were calculated as mean intensity (MI), mean abundance (MA), and prevalence (P%) (Bush et al., 1997). The infracommunity was characterized by the following indicators: total number of species, mean number of species, total number of specimens, mean number of specimens, Brillouin's diversity index, Pielou's evenness index, and Simpson's dominance index (Magurran, 1988).

Results

Parasitological research: In 2019-2021, 132, 14, and 6 roach specimens were examined from Kudelin, Novo Selo, and Koshava, respectively. An infection with a total of nine parasite species was found – *Allocreadium isoporum* (Looss, 1894) Looss, 1902; *Asymphylodora tincae* (Modeer, 1790) Lühe, 1909;

Table 1. Ecological indices of *Rutilus rutilus* parasites from the Danube River, biotope Kudelin (N – number of investigated fish; n – number of infected fish; p – number of fish parasites; MI – mean intensity; MA – mean abundance; P% – prevalence).

***Rutilus rutilus* (N = 132)**	**n**	**p**	**MI**	**MA**	**P%**	**Range**
Parasite species						
Allocreadium isoporum (Looss, 1894) Looss, 1902	2	4	2.00	0.03	1.52	1-3
Asymphylodora tincae (Modeer, 1790) Lühe, 1909	3	4	1.33	0.03	2.27	1-2
Palaeorchis incognitus Szidat, 1943	1	1	1.00	0.01	0.76	1
Caryophyllaeides fennica (Schneider, 1902) Nybelin, 1922	1	1	1.00	0.01	0.76	1
Proteocephalus torulosus (Batsch, 1786) Nufer, 1905	2	5	2.50	0.04	1.52	1-4
Acanthocephalus anguillae (Müller, 1780) Lühe, 1911	1	1	1.00	0.01	0.76	1
Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908	3	11	3.67	0.08	2.27	1-8
Philometra ovata (Zeder, 1803)	4	5	1.25	0.04	3.03	1-2
Rhabdochona denudata (Dujardin, 1845) Railliet, 1916	1	1	1.00	0.01	0.76	1

Table 2. Infracommunity of *Rutilus rutilus* from the Danube River, biotope Kudelin.

Number of *Rutilus rutilus* specimens	**Number of parasite species**		
	0	1	2
	117	12	3
Total number of species (Mean number of species ± SD)	9 (0.14 ± 0.41)		
Total number of specimens (Mean number of specimens ± SD)	33 (0.25 ± 0.98)		
Range	1–9		
Brillouin's diversity index (HB)	0.95 ± 0.24		
Pielou's evenness index (E)	0.79 ± 0.13		
Simpson's dominance index (C)	0.36 ± 0.14		

Palaeorchis incognitus Szidat, 1943; *Caryophyllaeides fennica* (Schneider, 1902) Nybelin, 1922; *Proteocephalus torulosus* (Batsch, 1786) Nufer, 1905; *Acanthocephalus anguillae* (Müller, 1780) Lühe, 1911; *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908; *Philometra ovata* (Zeder, 1803); *Rhabdochona denudata* (Dujardin, 1845) Railliet, 1916. The most parasite species (9 species) were found in the roach from Kudelin. One parasite species (*C. fennica*) was found in a roach from Koshava. Two parasite species (*As. tincae* and *P. incognitus*) were found in the roach from Novo Selo.

The component community and infracommunity of *R. rutilus* from the Kudelin biotope were considered, as the largest sample was collected there. Data on the species diversity and ecological indices of parasites in *R. rutilus* from the Koshava and Novo Selo biotopes were presented.

Component community of *R. rutilus* from the Danube River, Kudelin biotope: In the component community of roaches from the Danube River, Kudelin biotope, the largest number of specimens was represented by acanthocephalans (2 species with 12 specimens), followed by trematodes (3 species with nine specimens). An equal number of species and specimens (two species, each with six specimens) were found to have cestodes and nematodes. All found parasites were accidental species in the roach's component community. The highest mean intensity and mean abundance were observed in *P. laevis*, and the highest prevalence was found in *Ph. ovata* (Table 1).

Infracommunity of R. rutilus from the Danube River, Kudelin biotope: Of the collected and examined 132 specimens of roach from the Kudelin biotope, 117 specimens (88.64%) were not infected, and 15 specimens (11.36%) were infected. Twelve *R. rutilus* specimens were infected with one parasite species, and three *R. rutilus* specimens were infected with two parasite species. In the infracommunity of roaches from the Danube River (Kudelin), the number of parasites found varied from 1 to 9 in one *R. rutilus* specimen. Thirty-three parasite specimens were examined (Table 2).

Parasites of R. rutilus from the Danube River, Novo Selo, and Koshava biotopes: Fourteen specimens of roach from the Novo Selo biotope were examined, and

Table 3. Endoparasites of *Rutilus rutilus* from the Danube River and Danube River basin.

Authors	Localities	Species composition of the endoparasites of *Rutilus rutilus*
River Danube in other countries		
Stroe et al. (2021)	Romania	*Ligula intestinalis* (Linnaeus, 1758) Gmelin, 1790
Stroe et al. (2022)	Romania	*L. intestinalis*
River Danube in Bulgaria		
Kakacheva-Avramova et al. (1978)	Silistra, Vidin	*Bucephalus polymorphus* von Baer, 1827
	Ruse, Lom, Svishtov, Silistra	*Pr. torulosus*
Atanasov (2012)	Archar	*B. polymorphus*
	Gomotartsi	*Ph. ovata*
Kirin et al. (2013)	River Danube and Lake Srebarna	*Eustrongylides excisus* Jägerskiöld, 1909 (larvae)
Danube River Basin in other countries		
Lucký and Král (1983)	Korycany water reservoir, Czech Republic	*C. fennica*
	Ludkovice water reservoir, Czech Republic	*Nematoda* sp.
Hanzelová et al. (2009)	Latorica River, Slovakia	*P. incognitus*; *C. fennica*
Oros and Hanzelová (2009)	Tisa River, Slovakia	*C. fennica*
Djikanovic et al. (2011)	Danube River basin in Serbia	*All. isoporum*; *As. tincae*; *Sphaerostoma bramae* (Müller, 1776) Lühe, 1909; *C. fennica*; *Caryophyllaeus brachycollis* Janiszewska, 1953; *Pr. torulosus*; *Pseudocapillaria tomentosa* (Dujardin, 1845) Moravec, 1987; *Raphidascaris* sp.; *Ph. ovata*; *Philometra rischta* Skrjabin, 1917; *Rh. denudata*
Docan et al. (2021)	Prut River, Romania	*Caryophyllaeus fimbriceps* Annenkova-Chlopina, 1919; *L. intestinalis*
Danube River Basin in Bulgaria		
Shukerova (2010)	Srebarna Lake	*Pr. torulosus*; *Contracaecum microcephalum* (Rudolphi, 1809) (larvae); *Ac. anguillae*
Shukerova and Kirin (2019)	Srebarna Lake	*Pr. torulosus*; *C. microcephalum* (larvae); *Ac. anguillae*

only three fish hosts were infected. Two trematode species, viz. *As. tincae* and *P. incognitus*, were identified with two specimens each. Six *R. rutilus* specimens from the Koshava biotope were examined, and only one roach specimen was infected with one specimen of *C. fennica*.

Discussions

Several authors have investigated the parasite fauna of *R. rutilus* from the Danube River and its surrounding river basin (Table 3). In the present study, the ecological indices indicated by Hanzelová et al. (2009) and Oros and Hanzelová (2009) of *C. fennica* in roach from the Tisza River (MI = 3, P% = 23.9) and Latorica River (MI = 1.8, P% = 21.7), compared to those of the Danube River, Kudelin biotope (MI = 1.00, P% = 0.76) and Koshava biotope (MI = 1.00, P% = 16.67), are lower. Values for MI and P% for *P. incognitus* from the Latorica River (MI = 31, P% = 4.3) were compared with those of the Danube River, Kudelin biotope (MI = 1.00, P%=0.76), and Novo Selo biotope (MI = 1.00, P% = 14.29), and the MI in the present study for the Danube River (Kudelin and Novo Selo biotopes) is much lower, and P% from the Kudelin biotope is much higher (Hanzelová et al., 2009; Oros and Hanzelová, 2009). In contrast, the Novo selo biotope is lower than indicated for the roach trematode from the Latorica River. The values for MA and P% of *Ph. ovata* (MA = 0.09, P% = 2.13) in *R. rutilus* from the Danube River, Gomotartsi (Atanasov, 2012), compared with those of the present study from Kudelin biotope (MA = 0.04, P% = 3.03), revealed that the established value from Kudelin biotope for MA was lower, while the value for P% was higher.

The endoparasite species found in the present study have not been reported for the Danube River in other countries' territories. Of the endoparasites found in the

Table 4. Distribution of the endoparasites of *Rutilus rutilus* (" ● " – presence of the parasite species; "-" – absence of the parasite species).

Studies / Parasite species	Present study	River Danube in other countries	Danube River Basin in other countries	River Danube in Bulgaria	Danube River Basin in Bulgaria
Allocreadium isoporum	●	-	●	-	-
Asymphylodora tincae	●	-	●	-	-
Palaeorchis incognitus	●	-	●	-	-
Caryophyllaeides fennica	●	-	●	-	-
Proteocephalus torulosus	●	-	●	●	●
Acanthocephalus anguillae	●	-	-	-	●
Pomphorhynchus laevis	●	-	-	-	-
Philometra ovata	●	-	●	●	-
Rhabdochona denudata	●	-	●	-	-

present study: 1) seven endoparasite species have been reported for the species from the river basin in other countries; 2) two endoparasite species have been reported for roach from the Bulgarian section of the river; 3) two endoparasite species have been reported for *R. rutilus* from the Danube River basin in Bulgaria (Tables 3-4).

One of the nine species of endohelminths found in the present study, the acanthocephalan *P. laevis*, is pathogenic for fish. Acanthocephalans cause pomphorhynchosis, a disease that leads to delayed growth, impaired movement, and intestinal damage in fish and other organisms (Novakov et al., 2015). In the present study, it was found that *P. laevis* in roaches from the Kudelin biotope had the highest mean intensity (MI = 3.67) and mean abundance (MA = 0.08) among the eight endohelminth species found in this biotope. The ecological indices of the acanthocephalan *P. laevis* are recommended for monitoring as a fish pathogenic species. *Rutilus rutilus* is a new host record for *All. isoporum*, *As. tincae*, *P. incognitus*, *C. fennica*, *P. laevis,* and *Rh. denudata* in Bulgaria. Kudelin, Koshava, and Novo selo biotopes are new habitats for the endoparasites found in roaches. New data on the component communities and infracommunities of the roach from the Danube River were presented.

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