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PARASITE DIVERSITY OF *Cyprinus carpio* LINNAEUS 1758 FROM THE DANUBE RIVER, BULGARIA

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ABSTRACT

In 2019-2021, 20 specimens of common carp *Cyprinus carpio* Linnaeus 1758 were caught in three locations (biotopes) along the course of the Danube River in northwestern Bulgaria. Infection with five parasite species was detected – *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960 (class Trematoda); *Schyzocotyle acheilognathi* (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015 (class Cestoda); *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911, *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908 (class Acanthocephala); *Contracaecum* sp. (larvae) (class Nematoda). Three of the found endoparasite species are pathogenic for fish – *Sch. acheilognathi*, *P. laevis*, and *Contracaecum* sp. (larvae). *C. carpio* is a new host record for *Sch. acheilognathi* and *Ac. lucii* in Bulgaria. The studied biotopes are new habitats for the established parasite species. The present study aims to provide new data on the diversity and the ecological indices (MI, MA, P%) of *C. carpio* parasites from the Bulgarian section of the Danube River.

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INTRODUCTION

The Danube River rises from the Black Forest in Germany, flows in a west-east direction, crosses the territory of ten countries, and flows into the Black Sea (Pantelica et al., 2012). The river basin covers 10% of the territory of Europe (Gasparotti et al., 2013). The river basin is divided into the Upper Danube, Middle Danube, and Lower Danube (Schiemer et al., 2004). The Bulgarian section of the river lies between river kilometres 845 and 375 and is part of the Lower Danube (Polačik et al., 2008). The ichthyofauna of the Danube River basin comprises over 100 species, while 69 species have been reported for the Lower Danube (Ciolac, 2004). The Danube River provides conditions for the development of shipping, fishing, tourism, agriculture, and others (Enache et al., 2009; Gideon et al., 2013). Common carp *Cyprinus carpio* Linnaeus 1758 is a species of great economic importance (Yancheva et al., 2016). *C. carpio* is a freshwater, brackish, benthopelagic fish. It inhabits reservoirs with rich aquatic vegetation. Common carp is an omnivorous species. Its diet mainly includes zooplankton, benthic invertebrates, and aquatic vegetation (Karapetkova and Zhivkov, 2006; Froese and Pauly, 2024). *C. carpio* is included in the Red Book of the Republic of Bulgaria (category "CR=Critically Endangered") and the IUCN Red List (category "VU=Vulnerable") (Golemanski, 2011; IUCN, 2024). Fish and their parasites represent an essential part of aquatic ecosystems (Kirin, 2013; Kirin and Kuzmanova, 2014). Parasites are found at different trophic levels and food webs (Marcogliese, 2004; 2005). Few authors have studied the parasite fauna of common carp from the Danube River in other countries (Đikanović et al. 2018; Stroe et al., 2021; Stroe et al., 2022) and Bulgaria (Margaritov, 1966, 1975; Kakacheva-Avramova et al., 1978). Most studies on the parasite fauna of common carp are from the river basins in other countries (Lucký and Král, 1983; Molnár and Székely, 1995; Cojocaru, 2003; Hanzelová et al., 2009; Oros and Hanzelová, 2009; Djikanovic et al., 2011; Docan et al., 2019; Docan et al., 2021) and Bulgaria (Shukerova, 2006; 2010; Kirin et al., 2013). Urdeş and Hangan (2013) investigated the parasite fauna of common carp from the Danube Delta.

The purpose of the present study is to provide new data on the species diversity and the ecological indices (mean intensity (MI), mean abundance (MA), and prevalence (P%)) of *C. carpio* parasites from the Bulgarian section of the Danube River, falling into the Lower Danube.

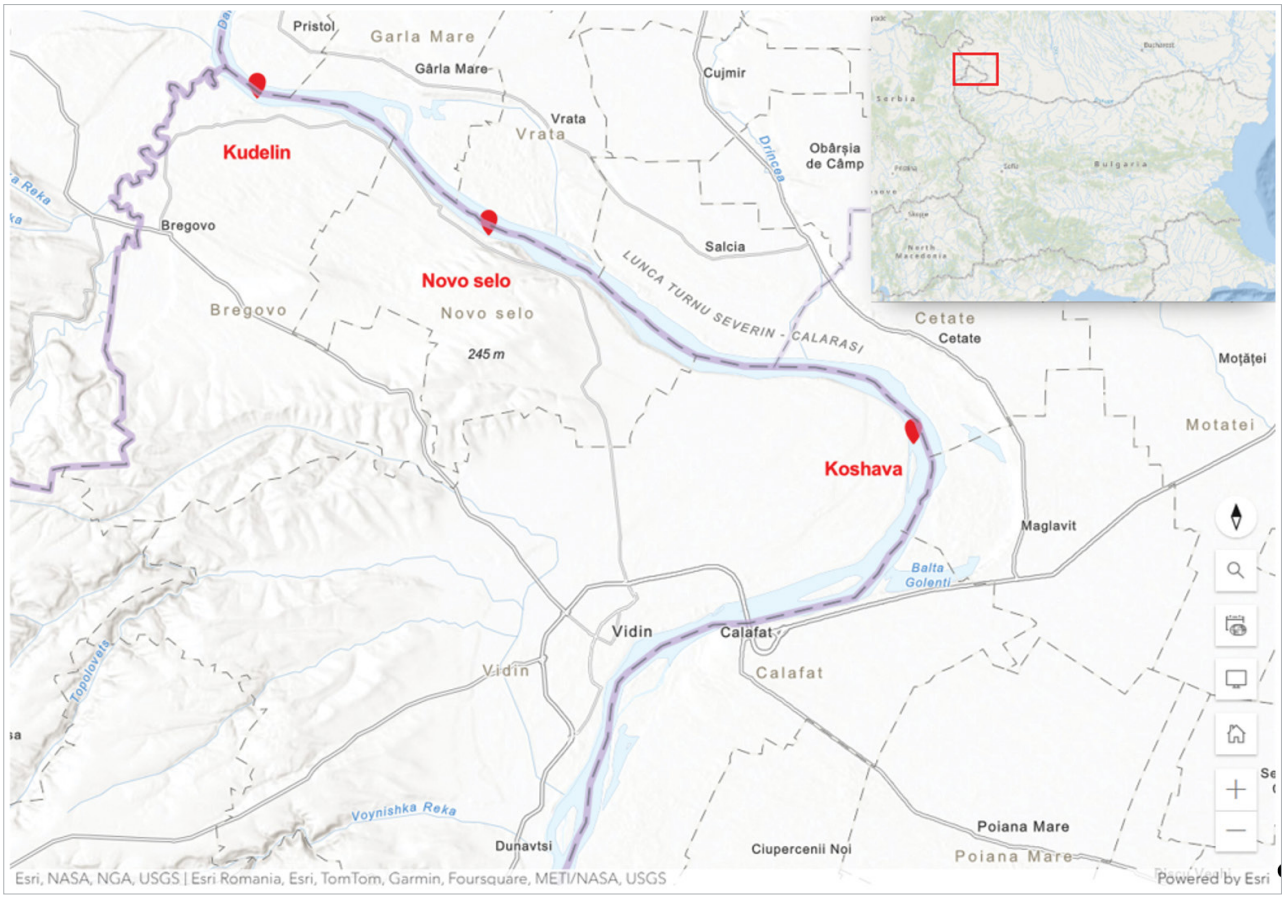

Fig 1. Kudelin, Novo selo and Koshava biotopes from the Danube River, Bulgaria (ArcGIS Online)

MATERIALS AND METHODS

The subject of the parasitological research were 12, 6, and 2 *C. carpio* specimens caught from 3 biotopes (Kudelin, Novo selo, and Koshava, respectively) along the Danube River after the river had entered Bulgarian territory. The Kudelin, Novo selo, and Koshava biotopes are located at river kilometre 844 (44°12'07.9"N 22°41'27.0"E), river kilometre 833 (44°08'56.0"N 22°48'56.0"E), and river kilometre 807 (44°03'32.3"N 23°02'02.8"E), respectively (Fig. 1).

The biotopes were visited in spring, summer, and autumn. The fish were caught in 2019-2021 with nets (gill nets with a mesh size of 2.8 cm and 3.2 cm; height 1.5 m; length 15 m) specified in the fishing permits for scientific purposes (Executive Agency for Fisheries and Aquaculture, Ministry of Agriculture and Food) and with fishing rods. The species' scientific name was given by Froese and Pauly (2024). In the field, caught specimens were weighed and measured. The weight of the fish was measured with a Kern EMB 500-1 laboratory balance, and the length was measured with a measuring line. Basic metric data was recorded – total body length (TL) in centimeters, maximum body height (MH) in centimeters, and weight (BW) in grams (Table 1).

After capture, the fish were killed by placing them in a container with clove oil (Fernandes et al., 2016), followed by dissection of the internal organs and examination for the presence of parasites. Twenty *C. carpio* specimens were examined for parasites using methods by Zashev and Margaritov (1966). A stereo microscope MZ1240 (Micros Austria) was used. Isolated parasites were fixed and stored in 70% ethyl alcohol. Temporary and permanent microscopic preparations were prepared to determine the discovered parasite species (Zashev and Margaritov, 1966; Moravec, 2013). For each parasite species, the ecological indices mean intensity (MI), mean abundance (MA), and prevalence (P%) were determined (Bush et al., 1997).

RESULTS AND DISCUSSION

During 2019-2021, 20 specimens of common carp from the Danube River (12 specimens from Kudelin, six specimens from Novo selo, and two specimens from Koshava) were subjected to ecoparasitological research. A total of five parasite species were isolated – *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960; *Schyzocotyle acheilognathi* (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015; *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911, *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908; *Contracaecum* sp. (larvae). Most parasite species (three) were found in common carp from the Novo selo biotope (Table 2).

Six species are free of parasites from the 12 specimens of *C. carpio* of the biotope Kudelin. Two parasite species were found (*Sch. acheilognathi* and *P. laevis*). Of these two, *P. laevis* had higher ecological indices. One parasite species was found in five of the examined specimens of common carp from Kudelin; in one of the specimens of common carp, two parasite species were found. The common carp specimens from the Kudelin biotope were collected in the autumn of 2020, in the spring and summer of 2021, with the highest infection in the spring due to more intensive feeding after the winter. During the examination of 6 specimens of common carp from Novo selo, it was found that all specimens (100%) were infected. The fish from the Novo selo biotope were caught in the summer of 2019. Three parasite species were found (*N. skrjabini*, *Ac. lucii*, and *Contracaecum* sp.). *Contracaecum* sp. had the highest values for MI, MA, and P%. One parasite species was found in four specimens of common carp from Novo selo; in one specimen of common carp, two parasite species were found. Two specimens of common carp were captured from Koshava, and only one was infected. Common carp from the Koshava biotope were caught only in the spring of 2019. One parasite species was found – *P. laevis*.

Table 1. Metric data of the studied *Cyprinus carpio* specimens

Danube River		TL (cm)	MH (cm)	BW (g)
Cyprinus carpio (N = 12) Kudelin	min – max	20 – 44	8 – 15	417 – 1980
	Mean ± SD	29.33 ± 12.86	10.67 ± 3.79	939.33 ± 901.25
Cyprinus carpio (N = 6) Novo selo	min – max	8.3 – 9	2.8 – 3	8 – 9
	Mean ± SD	8.73 ± 0.38	2.87 ± 0.12	8.67 ± 0.58
Cyprinus carpio (N = 2) Koshava	min – max	33 – 56	10 – 17	458 – 2460
	Mean ± SD	44.50 ± 16.26	13.50 ± 4.95	1459 ± 1415.63

Table 2. Species diversity of *Cyprinus carpio* parasites from the present study by biotope

Biotopes / Parasite species	Kudelin	Novo selo	Koshava
Nicolla skrjabini	-	+	-
Schyzocotyle acheilognathi	+	-	-
Acanthocephalus lucii	-	+	-
Pomphorhynchus laevis	+	-	+
Contracaecum sp.	-	+	-

"+" – presence of the parasite species

"-" – absence of the parasite species

Only *P. laevis* was found in two studied biotopes (Kudelin and Koshava). The prevalence of the parasite is the same in both biotopes, while the mean intensity and abundance in Koshava are higher than those in Kudelin (Table 3).

Several authors studied the parasite fauna of common carp from the Danube River and its river basin (Tables 4 a,b).

Of the parasites isolated in the present study, *N. skrjabini* and *P. laevis* were reported in common carp from the Danube River in Bulgaria. *Sch. acheilognathi*, *Ac. lucii*, and *Contracaecum* sp. were reported for *C. carpio* from the Danube River basin (Tables 4a, b-5).

In the present study, the ecological indices indicated by Shukerova (2006) – mean abundance (MA) and prevalence (P%) of *C. microcephalum* (MA=12.50, P%=35.71) in *C. carpio* from Lake Srebarna were compared with the values for MA and P% of *Contracaecum* sp. (MA=8.00, P%=66.67) from the Danube River, Novo selo biotope. In Novo selo, *Contracaecum* sp. has a lower mean abundance but a higher prevalence. Kirin et al. (2013) studied common carp from Lake Srebarna and did not detect infection with endohelminths.

Sch. acheilognathi, *P. laevis*, and *Contracaecum* sp. (larvae) are pathogenic to fish. The cestode *Sch. acheilognathi* causes botryocephalosis in carp and other fish species. The cestode can cause intestinal obstruction and perforation (Novakov et al., 2015). In fish infested with *Sch. acheilognathi*, an enlarged abdomen, slow movements, rapid emaciation and mass mortality are observed (Georgiev, 1995). The acanthocephalan *P. laevis* causes the pomphorhynchosis in some fish species, resulting in delayed growth, delayed movements, intestinal damage, and others (Novakov et al., 2015). In fish *Contracaecum* sp. larvae reduce the weight and lipid content of the liver. The high intensity of invasion leads to the destruction of the liver. Nematode larvae can kill fish if they enter the heart area. *Contracaecum* sp. larvae are also pathogenic to humans and cause anisakidosis when they enter the human body (Zashev and Margaritov, 1966; Demir and Karakişi, 2014).

Table 3. Species diversity and ecological indices in the component community of *Cyprinus carpio* (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)

***Cyprinus carpio* (Kudelin, N = 12)** **Parasite species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Schyzocotyle acheilognathi (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015	1	1	1.00	0.08	8.33	1
Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908	6	32	5.33	2.67	50.00	1-17
***Cyprinus carpio* (Novo selo, N = 6)** **Parasite species**						
Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960	1	1	1.00	0.17	16.67	1
Acanthocephalus lucii (Müller, 1776) Lühe, 1911	2	2	1.00	0.33	33.33	1
Contracaecum sp. (larvae)	4	48	12.00	8.00	66.67	2-20
***Cyprinus carpio* (Koshava, N = 2)** **Parasite species**						
Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908	1	40	40.00	20.00	50.00	40

Table 4a. Species composition of the endohelminths of *Cyprinus carpio* from the Danube River

Parasites of *Cyprinus carpio* from Authors	**Danube River in other countries**	**Danube River in Bulgaria**
Margaritov, 1966	-	*N. skrjabini; P. laevis*
Margaritov, 1975	-	*Ichthyocotylurus pileatus* (Ru-dolphi, 1802) Odening, 1969
Kakacheva-Avramova et al., 1978	-	*N. skrjabini; P. laevis*
Stroe et al., 2021	*Ligula intestinalis* (Linnaeus, 1758) Gmelin, 1790	-
Stroe et al., 2022	*L. intestinalis; Schulmanela* sp.	-

Table 4b. Species composition of the endohelminths of *Cyprinus carpio* from the Danube River basin

Parasites of *Cyprinus carpio* from Authors	**Danube River Basin in other countries**	**Danube River Basin in Bulgaria**
Molnár and Székely, 1995	*Caryophyllaeus fimbriceps* Annenkova-Chlopina, 1919; *Aspidogaster limacoides* Diesing, 1834	-
Molnár and Moravec, 1997	*Skrjabillanus cyprini* n. sp.	-
Cojocaru, 2003	*Caryophyllaeus laticeps* (Pallas, 1781) Lühe, 1910; *Schyzocotyle acheilognath*i (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015 (syn. *Bothriocephalus gowkongensis*)	-
Shukerova, 2006	-	*Contracaecum microcephalum* (Rudolphi, 1809) (larvae); *Raphidascaris acus* (Boch, 1779) (larvae); *Philometra cyprinirutili* (Creplin, 1825) Moravec, 2004 (syn. *Philometra cyprini*) *Paradilepis* sp.
Hanzelová et al., 2009	*C. laticeps; Ac. anguillae; P. tereticollis*	-
Oros and Hanzelová, 2009	*C. laticeps; Ac. anguillae; P. tereticollis*	-
Shukerova, 2010	-	*C. microcephalum* (larvae); *R. acus* (larvae); *Ph. cyprinirutili; Paradilepis* sp. (larvae)
Djikanovic et al., 2011	*Caryophyllaeus brachycollis* Janiszewska, 1953; *C. laticeps*; *Bathybothrium rectangulum* (Bloch, 1782) Lühe, 1902; *Pseudocapillaria tomentosa* (Dujardin, 1845) Moravec, 1987; *Hysterothylacium bidentatum* (Linstow, 1899) Deardorff & Overstreet, 1981; *Camallanus lacustris* (Zoega, 1776); *Neoechinorhynchus rutili* (Müller, 1780) Hamann in Stiles & Hassall, 1905; *Ac. anguillae*; *Ac. lucii*; *Acanthocephalus tenuirostris* (Achmerov & Dombrovskaja-Achmerova, 1941) Yamaguti, 1963; *Acanthocephalus* sp.	-
Docan et al., 2019	*Rhabdochona denudata* (Dujardin, 1845) Railliet, 1916; *N. rutili*	-
Docan et al., 2021	*C. fimbriceps*; *L. intestinalis*; *Schulmanela petruschewskii* (Shulman, 1948) Ivashkin, 1964	-

Table 5. Distribution of the found endoparasites (in the present study) of *Cyprinus carpio* from the Danube River and its basin

Studies	Present study	Danube River in other countries	Danube River basin in other countries	Danube River in Bulgaria	Danube River basin in Bulgaria
Parasite species					
Nicolla skrjabini	●			●	
Schyzocotyle acheilognathi	●		●		
Acanthocephalus lucii	●		●		
Pomphorhynchus laevis	●			●	
Contracaecum sp.	●				●

CONCLUSIONS

As a result of the parasitological examination of *C. carpio* from the Danube River, the Kudelin, Koshava, and Novo selo biotopes, infections with two (*Sch. acheilognathi*, *P. laevis*), one (*P. laevis*), and three (*N. skrjabini*, *Ac. lucii*, *Contracaecum* sp.) parasite species were found, respectively. All common carp specimens from the Danube River, Novo selo biotope, are infected due to the smaller size of the fish caught from this biotope and the presence of infected intermediate hosts. *C. carpio* is a new host record for *Sch. acheilognathi* and *Ac. lucii* in Bulgaria. The Kudelin, Koshava, and Novo selo biotopes are new habitats for the established parasite species. The presence of endohelminth species pathogenic for fish (*Sch. acheilognathi*, *P. laevis*, *Contracaecum* sp. larvae) and humans (*Contracaecum* sp. larvae) recommends more frequent helminthological examinations of carp and observations of the ecological indices of the parasites for the protection of carp populations, biodiversity in freshwater ecosystems and human health.

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RAZNOLIKOST PARAZITA KOD ŠARANA *Cyprinus carpio* LINNAEUS, 1758 IZ DUNAVA, BUGARSKA

SAŽETAK

U razdoblju 2019. – 2021. ulovljeno je 20 primjeraka jedinki šarana, *Cyprinus carpio* Linnaeus, 1758, na tri lokacije (biotopa) u rijeci Dunav u sjeverozapadnoj Bugarskoj. Utvrđena je infekcija s pet vrsta parazita – *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960 (razred Trematoda); *Schyzocotyle acheilognathi* (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015 (razred Cestoda); *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911, *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908 (razred Acanthocephala); *Contracaecum* sp. (ličinke) (razred Nematoda). Tri od pronađene vrste endoparazita za ribe su patogeni – *Sch. acheilognathi*, *P. laevis* i *Contracaecum* sp. (ličinke). *C. carpio* je novi domaćin za *Sch. acheilognathi* i *Ac. lucii* u Bugarskoj. Proučavani biotopi su nova staništa za etablirane vrste parazita. Cilj ove studije je pružiti nove podatke o raznolikosti i ekološkim indeksima (MI, MA, P%) parazita *C. carpio* iz bugarskog dijela rijeke Dunav.

Ključne riječi: endoparaziti, Koshava, Kudelin, Novo Selo, ekološki indeksi, patogene vrste

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