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HELMINTOLOGICAL STUDY OF FISH FROM THE FRESHWATER ECOSYSTEMS OF THE LUDA YANA RIVER AND CHEPELARSKA RIVER

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Abstract

In the autumn of 2022, an ecologohelinological study was carried out on 41 and 70 fish specimens, respectively, from the Luda Yana River and the Chepelarska River, falling into the Maritsa River basin in the territory of Bulgaria. The fish from the Luda Yana River were caught from three places (indicated as biotopes) - Popinitsi, Svoboda, and Chernogorovo, and belong to 3 species (Orpheus dace, Squalius orpheus Kottelat & Economidis, 2006; Round-scaled barbel, Barbus cyclolepis Heckel, 1837 and Bleak, Alburnus alburnus Linnaeus, 1758). The fish from the Chepelarska River were caught from one place - the Katunitsa biotope and belong to 4 species (Sq. orpheus, B. cyclolepis, Alb. alburnus, Vardar nase Chondrostoma vardarense Karaman, 1928). In both studied rivers, the dominant fish species caught is Sq. orpheus. The species diversity of the parasites and their ecological indicators are indicated for the non-dominant fish species. New habitats and new hosts have been discovered for some of the established helminth species.

Key words*: Bulgaria, Cyprinidae, ecological indices, helminths, Maritsa River Basin.*

INTRODUCTION

The Luda Yana River and the Chepelarska River are among the major tributaries of the Maritsa River on Bulgarian territory. The Luda Yana River (with a length of 74 km) rises from Sredna Gora Mountain, enters the Upper Thracian Plain, and flows from the left into the Maritsa River, north of the village of Sinitovo. The Chepelarska River (with a length of 86 km) rises from the Rhodope Mountain, enters the Upper Thracian Plain (in the region of the town of Assenovgrad), and flows from the right into the Maritsa River between the town of Plovdiv and the town of Sadovo (East Aegean River Basin Directorate, 2018). Three (Orpheus dace, *Squalius orpheus* Kottelat & Economidis, 2006; round-scaled barbell, *Barbus cyclolepis* Heckel, 1837 and bleak, *Alburnus alburnus* Linnaeus, 1758) of the four fish species subject to helminthological research in the present study are included in the IUCN Red List with the "LC" category. One species (*Chondrostoma vardarense* Karaman, 1928) is in the "NT" category. *B. cyclolepis* is protected by the Biological Diversity Act and the Habitats Directive. *Sq. orpheus*, *B. cyclolepis* and *Ch. vardarense* are endemic to Europe (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, 1992; Biological Diversity Act, 2002; Freyhof & Brooks, 2011; IUCN, 2024). Scientific studies on the species composition, the parasite ecological indices, and the parasite communities of fish from the Luda Yana River are scarce (Kirin, 2002b; Kirin et al., 2019; Zaharieva, 2023a). At present, the parasite fauna of fish from the Chepelarska River has been studied by Kakacheva-Avramova (1965), Kirin (2002a), Chunchukova (2020), and Zaharieva (2023b).

The purpose of the present study is to update the existing and provide new data on the helminth species diversity and the structure of helminth communities of fish from the freshwater ecosystems of the Luda Yana River and Chepelarska River, flowing into the Maritsa River on the territory of Bulgaria.

MATERIALS AND METHODS

A total of 111 specimens of fish from 4 species (Orpheus dace, *Squalius orpheus* Kottelat & Economidis, 2006; round-scaled barbell, *Barbus cyclolepis* Heckel, 1837; bleak, *Alburnus alburnus* Linnaeus, 1758; *Chondrostoma vardarense* Karaman, 1928), caught from two rivers flowing into the basin of the Maritsa River, were subjected to helminthological examination (Table 1).

Table 1. Caught fish specimens from the Luda Yana River and the Chepelarska River

Fish species	Luda Yana River N = 41	Chepelarska River N = 70
Squalius orpheus Kottelat & Economidis, 2006	N = 34	N = 51
Barbus cyclolepis Heckel, 1837	N = 5	N = 16
Alburnus alburnus (Linnaeus, 1758)	N = 2	N = 2
Chondrostoma vardarense Karaman, 1928	-	N = 1

For the research, three places (designated as biotopes) along the course of the Luda Yana River were visited - in the vicinity of the village of Popintsi, the village of Svoboda, and the village of Chernogorovo. Popintsi biotope is located along the course of the Luda Yana River before it enters the village of Popintsi (42°25'26.8"N 24°16'02.5"E; Panagyurishte Municipality; Pazardzhik Province). Svoboda biotope is located next to the bridge over the Luda Yana River on the road between the villages Svoboda and Popintsi, shortly before the Radka mine (42°24'07.3"N 24°17'24.2"E; Strelcha Municipality; Pazardzhik Province). Chernogorovo biotope is located next to the bridge over the Luda Yana River, southwest of the village of Chernogorovo on the road in the direction of the town of Pazardzhik (42°16'12.9"N 24°23'33.3"E; Pazardzhik Municipality; Pazardzhik Province) (Figure 1).

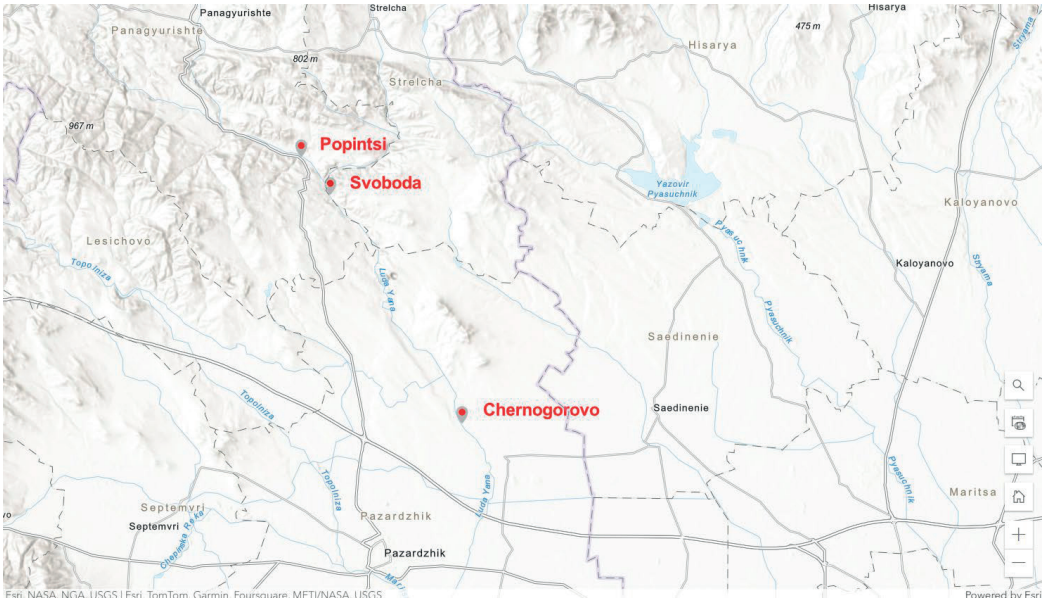

Figure 1. Researched biotopes from the Luda Yana River (https://www.esri.com/en-us/arcgis/products/arcgis-online/overview)

In connection with the study, two places were visited along the Chepelarska River - in the vicinity of the villages of Bachkovo and Katunitsa. Katunitsa biotope is located along the Chepelarska River in the western outskirts of the village of Katunitsa between the railway bridge and the concrete bridge (42°06'08.3"N 24°51'58.8"E; Sadovo Municipality; Plovdiv

Province). Bachkovo biotope includes two places located along the river between the village of Bachkovo and the town of Assenovgrad (41°57'52.5"N 24°51'57.0"E and 41°57'38.0"N 24°51'47.5"E; Assenovgrad Municipality; Plovdiv Province). Fish populations were not detected from the Bachkovo biotope during the study period (Figure 2).

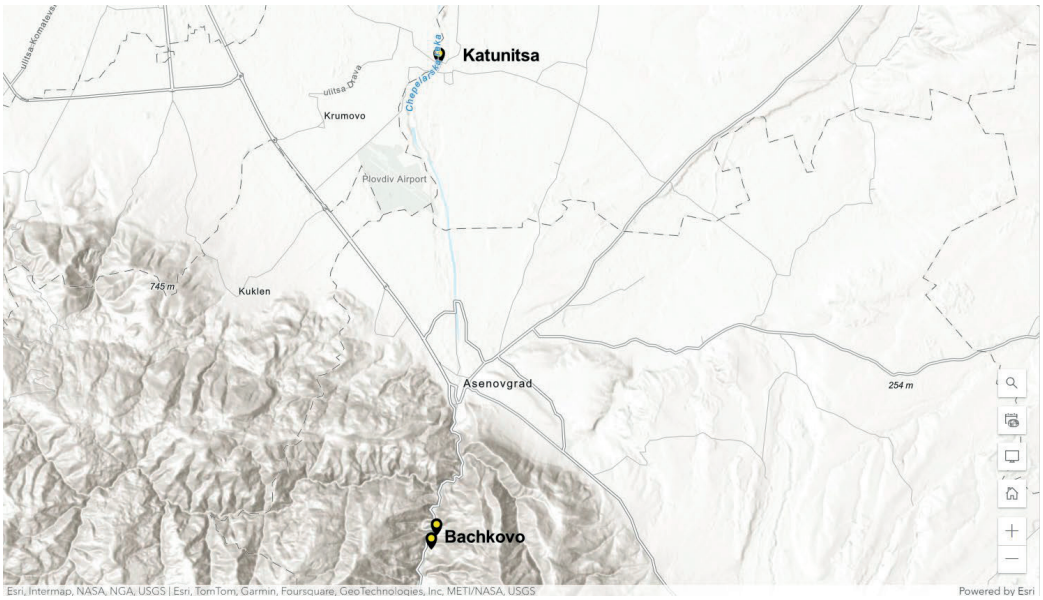

Figure 2. Researched biotopes from the Chepelarska River
(https://www.esri.com/en-us/arcgis/products/arcgis-online/overview)

The fish were caught according to the requirements of the Ministry of Agriculture and the Executive Agency for Fisheries and Aquaculture (EN 14757:2015). The catching of the fish was carried out after issuing a license to catch fish for scientific research purposes and a ticket for amateur fishing. The fish species was determined in the field (according to Karapetkova & Zhivkov, 2006; Froese & Pauly, 2023) and basic metric data (total body length - TL in centimeters; maximum body height - MH in centimeters; body weight - BW in grams) were recorded. The largest number of fish specimens were caught from the Katunitsa and Popintsi biotopes (Table 2).

The fish subjected to helminthological examination were collected in the autumn of 2022.

Table 2. Metric data of caught fish specimens by biotopes

River	Biotope	Fish species	TL (mean ± SD)	MH (mean ± SD)	BW (mean ± SD)
Luda Yana	Popintsi	*Squalius orpheus* (N = 32)	13.16 ± 3.22	2.71 ± 0.76	20.29 ± 12.38
	Svoboda	*Squalius orpheus* (N = 1)	6.3	1.3	2
		Barbus cyclolepis (N = 5)	9.1 ± 0.6	1.82 ± 0.23	5.6 ± 2.07
		Alburnus alburnus (N = 2)	10.2 ± 0.14	2.05 ± 0.07	6 ± 0.00
	Chernogorovo	*Squalius orpheus* (N = 1)	9.2	1.7	6
Chepelarska	Katunitsa	*Squalius orpheus* (N = 51)	13.74 ± 3.29	2.86 ± 0.74	24.64 ± 14.92
		Barbus cyclolepis (N = 16)	9.13 ± 4.64	2.23 ± 0.47	11.93 ± 8.05
		Alburnus alburnus (N = 2)	12.6 ± 0.28	2.6 ± 0.42	14 ± 2.83
		Chondrostoma vardarense (N = 1)	17	3.6	42
	Bachkovo	-	-	-	-

Immediately after catching 111 fish specimens, the body surface, the abdominal cavity, and the internal organs were examined for parasites. The collected host material samples were preserved in 70% ethyl alcohol and prepared for transport and subsequent laboratory processing. The helminthological examination was carried out according to standard methods (Zashev & Margaritov, 1966; Moravec, 2013; and others). For species identification of helminths, temporary microscopic preparations of the representatives of classes Acanthocephala and Nematoda (Zashev & Margaritov, 1966; Moravec, 2013) and permanent microscopic preparations of classes Trematoda and Cestoda (Georgiev et al., 1986; Scholz & Hanzelova, 1998) were prepared. The species of the isolated helminths were determined according to Bauer (Ed.) (1987), Moravec (2013), and others. Basic ecological indices were calculated, such as the number of infected fish and fish parasites, mean intensity, mean abundance, and prevalence (Bush et. al, 1997).

RESULTS AND DISCUSSIONS

Structure of the helminth communities

During the examination of a total of 111 fish specimens from the Luda Yana River and Chepelarska River, 12 helminth taxa (*Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960; *Caryophyllaeus brachycollis* Janiszewska, 1953; *Caryophyllaeides fennica* (Schneider, 1902) Nybelin, 1922; *Acanthocephalus anguillae* (Müller, 1780) Lühe, 1911; *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911; *Acanthocephalus tenuirostris* (Achmerov & Dombrovskaja-Achmerova, 1941) Yamaguti, 1963); *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908; *Contracaecum* sp.; *Schulmanela petruschewskii* (Schulman, 1948) Ivashkin, 1964; *Philometra ovata* (Zeder, 1803); *Raphidascaris acus* (Boch, 1779); *Rhabdochona denudata* (Dujardin, 1845) Railliet, 1916), belonging to 4 classes (Trematoda, Cestoda, Acanthocephala, Nematoda) were found (Table 3).

Table 3. Taxonomic position, fish host, and biotope of the established helminth specimens

Helminth	Taxonomic position	Fish host	Biotope
Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960	CLASS TREMATODA RUDOLPHI, 1808 Family Opecoelidae Ozaki, 1925 Genus *Nicolla* Višniewski, 1933	*Barbus cyclolepis*	Svoboda
Caryophyllaeus brachycollis Janiszewska, 1953	CLASS CESTODA RUDOLPHI, 1808 Family Caryophyllaeidae Leuckart, 1878 Genus *Caryophyllaeus* Müller, 1878	*Barbus cyclolepis*	Katunitsa
Caryophyllaeides fennica (Schneider, 1902) Nybelin, 1922	CLASS CESTODA RUDOLPHI, 1808 Family Lytocestidae Wardle and McLeod, 1952 Genus *Caryophyllaeides* Nybelin, 1922	*Squalius orpheus*	Popintsi
		Barbus cyclolepis	Svoboda
Acanthocephalus anguillae (Müller, 1780) Lühe, 1911	CLASS ACANTHOCEPHALA (RUDOLPHI, 1808) Family Echinorhynchidae (Cobbold, 1879) Hamann, 1892 Genus *Acanthocephalus* Koelreuther, 1711	*Squalius orpheus*, *Barbus cyclolepis*	Katunitsa
Acanthocephalus lucii (Müller, 1776) Lühe, 1911		*Squalius orpheus*	Popintsi
Acanthocephalus tenuirostris (Achmerov & Dombrovskaja-Achmerova, 1941) Yamaguti, 1963)		*Squalius orpheus*, *Barbus cyclolepis*	Katunitsa
Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908	CLASS ACANTHOCEPHALA (RUDOLPHI, 1808) Family Pomphorhynchidae Yamagiti, 1939 Genus *Pomphorhynchus* Monticelli, 1905	*Squalius orpheus*	Chernogorovo
Contracaecum sp.	CLASS NEMATODA RUDOLPHI, 1808 Family Anisakidae Skrjabin et Karokhin, 1945 Genus *Contracaecum* Railliet & Henry, 1912	*Squalius orpheus*	Katunitsa

Helminth	Taxonomic position	Fish host	Biotope
Schulmanela petruschewskii (Schulman, 1948) Ivashkin, 1964	CLASS NEMATODA RUDOLPHI, 1808 Family Capillariidae Railliet, 1915 Genus *Schulmanela* Ivashkin, 1964	*Barbus cyclolepis*	Katunitsa
Philometra ovata (Zeder, 1803)	CLASS NEMATODA RUDOLPHI, 1808 Family Philometridae Baylis et Daubney, 1926 Genus *Philometra* Costa, 1845	*Barbus cyclolepis*	Katunitsa
Raphidascaris acus (Boch, 1779)	CLASS NEMATODA RUDOLPHI, 1808 Family Raphidascarididae Genus *Raphidascaris* (Bloch, 1779) Railliet et Henry, 1915	*Alburnus alburnus*	Svoboda
		Squalius orpheus, *Barbus cyclolepis*	Katunitsa
Rhabdochona denudata (Dujardin, 1845) Railliet, 1916	CLASS NEMATODA RUDOLPHI, 1808 Family Rhabdochonidae Travassos, Artigas et Pereira, 1928 Genus *Rhabdochona* Railliet, 1916	*Squalius orpheus*	Popintsi
		Squalius orpheus	Katunitsa

Helminth communities of Squalius orpheus from the Luda Yana River and the Chepelarska River

During the examination of 32, 1, and 1 *Sq. orpheus* specimen from Popintsi biotope, Chernogorovo biotope, and Svoboda biotope, located along the Luda Yana River, a total of 4 helminth taxa were found - *C. fennica*, *Ac. lucii*, *Rh. denudata* and *P. laevis*. The studied *Sq. orpheus* specimen from Svoboda biotope was not infected. In the component community of Orpheus dace from Popintsi biotope, the representatives of class Acanthocephala (1 species with 15 specimens) had the largest number of specimens. They were followed by the representatives of class Nematoda (1 species with three specimens) and Cestoda (1 species with one specimen). One component helminth species (*Ac. lucii*) and two accidental helminth species (*C. fennica* and *Rh. denudata*) were found in the helminth community of *Sq. orpheus* from Popintsi biotope. For Popintsi biotope, *Ac. lucii* had the highest value for mean intensity (MI) and mean abundance (MA) (Figure 3).

During the ecologohelminthological examination of 51 *Sq. orpheus* specimens from Katunitsa biotope, located along the Chepelarska River, an infection with a total of 5 endohelminth species was found - *Ac. anguillae*, *Ac. tenuirostris*, *Contracaecum* sp., *R. acus*, l. and *Rh. denudata*. In the component community of *Sq. orpheus* from Katunitsa biotope, the largest number of specimens had the nematodes (3 species with 38 specimens), followed by the acanthocephalans (2 species with 4 specimens). In the component community of *Sq. orpheus*, one core species (*Contracaecum* sp.) was found, while the remaining helminth species were accidental. *R. acus* had the highest value for MI. *Contracaecum* sp. had the highest value for MA (Figure 3).

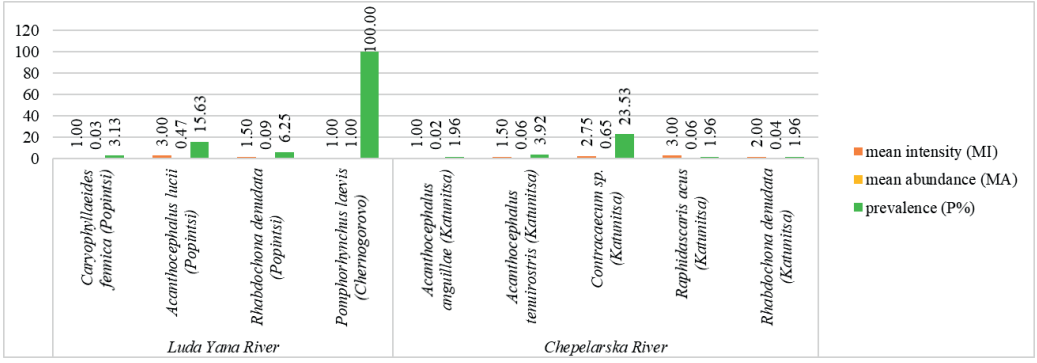

Figure 3. Ecological indices (MI, MA, P %) of *Sq. orpheus* helminths from the Luda Yana River and the Chepelarska River

There are few ecologohelminthological studies of *Sq. orpheus* from the Luda Yana River and the Chepelarska River.

The present article builds on and updates the existing data on the helminth fauna of *Sq. orpheus* from the two rivers, part of the Maritsa River Basin in Bulgaria (Zaharieva, 2023a, 2023b). Kirin et al. (2019) studied *Rutilus rutilus* Linaeus, 1758 from the Luda Yana River (Popintsi biotope) and reported the same three endohelminths species (*C. fennica*; *Ac. lucii* and *Rh. denudata*).

Chernogorovo biotope is a new habitat for *P. laevis* from *Sq. orpheus*. Kirin (2002a) reported *B. rectangulum*, *Ac. anguillae*, *Ac. tenuirostris*, *C. microcephalum* (l.) and *Rh. denudata* for *Sq. orpheus* from the Chepelarska River (between Assenovgrad and Bachkovo). Katunitsa biotope is a new habitat for *Ac. anguillae* and *R. acus* (l.) from Orpheus dace. *R. acus* (l.) is reported for the first time from *Sq. orpheus* from the Chepelarska River.

Helminth communities of Barbus cyclolepis from the Luda Yana River and the Chepelarska River

As a result of the ecologoparasitological examination of 5 *B. cyclolepis* specimens from Svoboda biotope, located along the Luda Yana River, two helminth species were found - one specimen each of *N. skrjabini* and *C. fennica*. A mixed infection was not found. In the component community of round-scaled barbell from the Svoboda biotope, the two established helminth species had equal values of the ecological indices (Figure 4).

In the current helminthological study of 16 *B. cyclolepis* specimens from Katunitsa biotope from the Chepelarska River, infection with six endohelminth species was found - *C. brachycollis*, *Ac. anguillae*, *Ac. tenuirostris*, *Ph. ovata*, *R. acus* (adult and l.), *Sch. petruschewskii*. In round-scaled barbell from Katunitsa biotope, *R. acus* had the highest ecological indices (Figure 4).

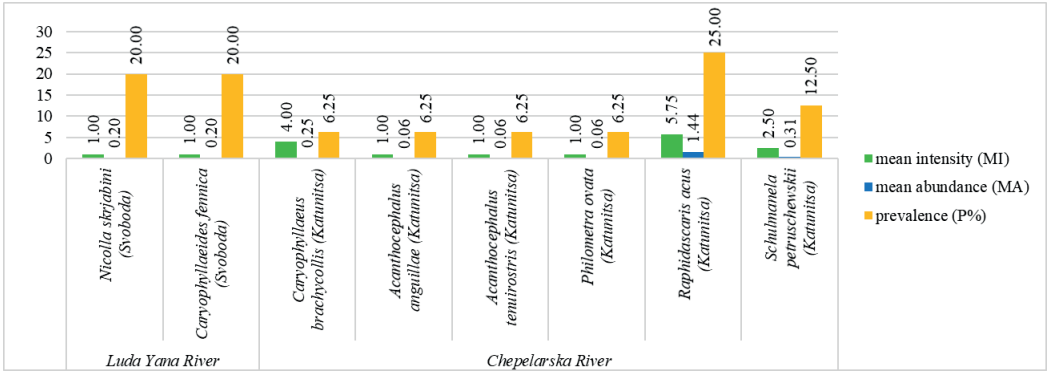

Figure 4. Ecological indices (MI, MA, P %) of *B. cyclolepis* helminths from the Luda Yana River and the Chepelarska River

Kirin (2002b) investigated *B. cyclolepis* (syn. *Barbus tauricus cyclolepis*) from the Luda Yana River (Panagyurishte biotope) and reported four helminth species - *All. isoporum*, *C. brachycollis*, *B. rectangulum*, *Ac. anguillae*. Svoboda biotope (Luda Yana River) is a new habitat for the two helminth species (*N. skrjabini* and *C. fennica*) from *B. cyclolepis* that are established in the present study. Katunitsa biotope is a new habitat for the six endohelminth species found in *B. cyclolepis*. *N. skrjabini*, *Ac. tenuirostris*, *Ph. ovata* and *R. acus* are reported for the first time from *B. cyclolepis* and the Aegean Water Basin in Bulgaria.

Helminth communities of Alburnus alburnus from the Luda Yana River and the Chepelarska River, and Chondrostoma vardarense from the Chepelarska River

During the helminthological examination of two *Alb. alburnus* specimens from the Svoboda biotope (Luda Yana River) found that only one specimen was infected. One helminth species was found (*R. acus*) with ecological indices: MI = 1.00; MA = 0.50; P% = 50.00.

Until now, there have been no ecoparasitological studies on *Alb. alburnus* from the Luda Yana River. Svoboda biotope is a new habitat for *R. acus* from bleak. *R. acus* is reported for the first time from *Alb. alburnus* from the Aegean Water Basin in Bulgaria. The examined two *Alb. alburnus* specimens and one *Ch. vardarense* specimen from Katunitsa biotope (Chepelarska River) were not infected.

CONCLUSIONS

For the current helminthological study, three biotopes were visited, located along the Luda Yana River, and three fish species (*Sq. orpheus*, *B. cyclolepis*, *Alb. alburnus*) were caught. Infection with a total of 6 endohelminth species (*N. skrjabini*, *C. fennica*, *A. lucii*, *P. laevis*, *R. acus*, *Rh. denudata*) was found. In addition, two biotopes were visited, located in the lower reaches of the Chepelarska River, and fish were caught only from Katunitsa biotope. Four fish species (*Sq. orpheus*, *B. cyclolepis*, *Alb. alburnus*, *Ch. vardarense*) were collected and infection with a total of 8 endohelminth species (*Ac. anguillae*, *Ac. tenuirostris*, *C. brachycollis*, *Contracaecum* sp., *Ph. ovata*, *R. acus*, *Rh. denudata*, *Sch. petruschewskii*) was found. In the helminthological studies on fish from both rivers, the highest species diversity of helminths was found in *Sq. orpheus*.

As a result of the research, the following conclusions can be made:

- ✓ Chernogorovo biotope (Luda Yana River) is a new habitat for *P. laevis* from *Sq. orpheus*.
- ✓ Svoboda biotope (Luda Yana River) is a new habitat for *N. skrjabini* and *C. fennica* from *B. cyclolepis*.
- ✓ Svoboda biotope (Luda Yana River) is a new habitat for *R. acus* from *Alb. alburnus*.
- ✓ Katunitsa biotope (Chepelarska River) is a new habitat for *Ac. anguillae* and *R. acus* (l.) from *Sq. orpheus*.
- ✓ Katunitsa biotope (Chepelarska River) is a new habitat for *C. brachycollis*, *Ac. anguillae*, *Ac. tenuirostris*, *Ph. ovata*, *R. acus* (adult and l.) and *Sch. petruschewskii* from *B. cyclolepis*.
- ✓ N. *skrjabini*, *Ac. tenuirostris, Ph. ovata*, and *R. acus* are reported for the first time for *B. cyclolepis* from the Aegean Water Basin in Bulgaria
- ✓ *R. acus* is reported for the first time for *Alb. alburnus* from the Aegean Water Basin in Bulgaria.
- ✓ *R. acus* (l.) is reported for the first time for *Sq. orpheus* from Chepelarska River.

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