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# BIODIVERSITY AND HELMINTH COMMUNITIES OF *Barbus cyclolepis* Heckel, 1837 FROM CHERNA RIVER, BULGARIA

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***Abstract***

*The study presents for the first time the Cherna River, southern Bulgaria, Aegean water basin, the results of research on the biological diversity and helminth communities of the Round-scaled barbell Barbus cyclolepis Heckel, 1837. 30 specimens of B. cyclolepis are studied. Infection by 5 species of helminths are found (Allocreadium isoporum (Loos, 1894); Bathybothrium rectangulum (Bloch, 1782); Caryophyllaeus laticeps (Pallas, 1781); Capillaria petruschewskii Zeder, 1800; Neoechinorhynchus rutili (Müller, 1780)). The infection indices and the dominant structure of helminth communities are presented. Basic biotic indices are determined. Helminth communities are analyzed at two levels: infracommunity and component community. All established parasite species are autogenous for the helminth communities of the Round-scaled barbell from the freshwater ecosystem of the Cherna River. New data for helminths and helminth communities of B. cyclolepis are presented. The bioindicator significance of helminths and helminth communities are discussed.*

***Key words:*** *bioindication, Barbus cyclolepis, Cherna River, helminth communities.*

## INTRODUCTION

Cherna River is distinguished by its exceptional biological diversity. It springs 5 km southeast of Mugla village, Smolyan municipality (1770 m above sea level). The river flows entirely on the territory of the Smolyan region. It flows into the village of Leshtak (Madan municipality) as a left tributary of the Arda River (624 m above sea level). Along its entire length, it flows in an eastern direction in a narrow canyon-like valley with a single valley extension in the area of the town of Smolyan. The river ecosystem is not subjected to intense negative anthropogenic impacts. Cherna River falls into the protected area BG0001030 Rhodope-Western, declared under the Habitats Directive (Directive 92/43/EEC) and protected area BG0002113 Trigrad - Mursalitsa, declared under the Birds Directive (Directive 79/409/EEC). Helminths are characterized by complex development, often involving more than one type of host. Therefore, the reduction of helminth infections and helminth diversity species is often reduced in the species diversity and population size of a number of species of free-living organisms (Thompson et al., 2016; Kevin & Lafferty, 2012). According to some scientific studies, parasites influence the behavior of the hosts and their health status (Preston & Johnson, 2010). Parasites and parasite communities are of fundamental ecological importance because they influence trophic relationships, food chains and webs, and biodiversity, especially for keystone species. The increase in parasite populations, especially in some species, has also been linked to impacts on host health, including and the human (Preston & Johnson, 2010; Zaharieva & Zaharieva, 2020a, b; Zaharieva & Zaharieva, 2020c,d; Zaharieva & Zaharieva, 2021a; Zaharieva & Zaharieva, 2021b). Parasites are an essential part of the elements of biological diversity, but at the same time, both their diversity and their communities are not well studied (Selbach et al., 2020). Parasites are also biological elements for bioindication and assessment of the state of the environment (degradation, pollution, loss of biodiversity, etc.) (Nachev & Sures, 2016; Vidal-Martinez & Wunderlich, 2017; Zaharieva & Zaharieva, 2021a; Zaharieva & Zaharieva, 2021b). Parasites and parasite communities of *B. cyclolepis* were studied by a few authors in Bulgaria (Kirin, 2002, 2003; Kakacheva-Avramova, 1965, 1972; Margaritov, 1965; Chunchukova, 2020; Chunchukova et al., 2020; Kirin et al., 2020, etc.). There are also few studies from other countries about parasites and parasite communities of pound-scaled barbel from the Aegean Water basin (Bazsalovicsová et

al., 2014, etc.). They refer mainly to representatives of the class Monogenea (Simkova et al., 2007; Benovics et al., 2018; Řehulková et al., 2020, etc.). Cherna River has not been the subject of systematic ecological, ecologoparasitological, and biomonitoring studies with the biological element endoparasites. The present research aims to present the biological diversity of the parasites of *B. cyclolepis* from the Cherna River, the structure of the component, and the infracommunities formed by them.

## MATERIALS AND METHODS

In 2019, a total of 30 specimens *Barbus cyclolepis* Heckel, 1837 are examined for helminths. The scientific and common names of the fish are presented according to the FishBase database (Fröse & Pauly, 2022). Helminthological examinations are conducted following research methods described by Petrochenko, 1956; Zashev & Margaritov, 1966; Bauer, 1987; Moravec, 2013. Helminth specimens were fixed and preserved in Eppendorf tubes with 70% ethylalcohol. The specimens of classes Trematoda and Cestoda are studied by methods of Georgiev et al., 1986; Scholz & Hanzelová, 1998 and of Acanthocephala and Nematoda - by methods of Zashev & Margaritov, 1966; Moravec, 2013. Analyses of the helminth community structure have been implemented in both levels: infracommunity (total and mean number of species; total and mean number of specimens; Brillouin's index of diversity (HB)) and component community (prevalence (P%) and mean intensity (MI) for each species) (Bush et al., 1997; Kennedy, 1993, 1997; Magurran, 1988). The species are divided into core species (P%>20), component species (P%>10), and accidental species (P%<10) (Kennedy, 1993). The diversity measures are calculated by software products Statistica 10 (StatSoft Inc., 2011) and MS Excel (Microsoft 2010).

## RESULTS AND DISCUSSIONS

Model fish species Round-scaled barbell (*Barbus cyclolepis* Heckel,1837; Cyprinidae) is a fresh water, benthopelagic and subtropical fish, distributed in Europe and Asia - Aegean Water Basin (Bulgaria, Turkey, and Greece), Black Sea Basin, etc. (Kottelat & Freyhof, 2007; Karapetkova & Zhivkov, 2009). In Bulgaria, the species is widespread in Maritsa, Mesta, and Struma rivers as well as its tributaries (Karapetkova & Zhivkov, 2009). The fish inhabits streams, lakes, and upper and middle streams of the fast-flowing rivers, but prefers areas with clear water and sandy-gravel bottom (Bianco, 1998; Kottelat & Freyhof 2007; Karapetkova & Zhivkov, 2009). It reaches a maximum length of up to 30 cm and a weight of up to 1000 g (Karapetkova & Zhivkov, 2009), as mean body length in decreasing age groups, sex varies in different rivers and habitats (Marinov, 1986; Dikov et al., 1994; Vasiliou & Economidis, 2005; Rozdina, 2009; Raikova & Kolev, 2015; Kolev, 2019; Çelik & Özuluğ, 2021).

The development of *B. cyclolepis* from rivers in Bulgaria was studied by Mihaylova, 1965; Marinov, 1986; Vasiliou & Economidis, 2005; Rozdina, 2009; Raikova-Petrova & Rozdina, 2012; Kolev & Raikova, 2019, etc.

In the food spectrum, from 14 food components at trophic systems of Round-scaled barbel from the middle part of the Maritsa River, the highest prevalence and index of dominance were determined for chironomid larvae as well as for plant detritus but mainly during the summer season (Rozdina et al., 2008). In Istranca Stream (Istanbul, Turkey), from 11 food components, the dominant Insects and fish was determined as selective to Diptera, reported by many other authors (Saç et al., 2021). *B. cyclolepis* is included in IUCN Red List as a Least Concern (LC) species (Bianco, 1998; Crivelli, 2006; Rozdina et al., 2008). For Bulgaria and Balkan Peninsula *B.cyclolepis* is an endemic fish species (Rozdina et al., 2008; Raikova & Kolev, 2015).

### Helminths and helminth community structure

As a result of the ecologoparasitological studies carried out on 30 specimens of the Round-scaled barbel (*B. cyclolepis*), infection with 5 species of parasites was found: *Allocreadium isoporum* (Loos, 1894); *Bathybothrium rectangulum* (Bloch, 1782); *Caryophyllaeus laticeps* (Pallas, 1781); *Schulmanela petruschewskii* (Schulman, 1948) Ivashkin, 1964; *Neoechinorhynchus rutili* (Müller, 1780). The identified parasite species

belong to 4 orders: Trematoda, Cestoda, Nematoda, and Acanthocephala (Table 1). *All. isoporum* parasitizes the intestines of family Cyprinidae fishes. The life cycle includes a first (*Sphaerium* Scopoli, 1777) and a second intermediate host (*Ephemera* Linnaeus, 1758, *Anabolia* Stephens, 1837, *Chaetopterix* Cuvier, 1827, larvae). *B. rectangulum* is a specific helminth species of *Barbus barbus* (Linnaeus, 1758) and *B. petenyi* Heckel, 1852. *C. laticeps* is an intestinal cyprinid helminth with intermediate hosts *Tubifex tubifex* (Müller, 1774), *T. barbatus* Grube, 1891, *Limnodrilus claparedeanus* Ratzel, 1868. *Sch. petruschewskii* infected the liver of freshwater fish (*Gymnocephalus cernua* (Linnaeus, 1758), *Cobitis taenia* Linnaeus, 1758, *Lepomis gibbosus* (Linnaeus, 1758), *Sander lucioperca* (Linnaeus, 1758), *Perca fluviatilis* Linnaeus, 1758, etc.). Intermediate host is *Eiseniella tetraedra* (Savigny, 1826). *N. rutili*is an intestinal parasite of freshwater fish species from the families Cyprinidae, Salmonidae, Esocidae, Percidae, Gobiidae, Cottidae, etc. Intermediate hosts are the species of insects *Sialis fulinginosa* Pictet, 1836 and *Apogonniger* Döderlein, 1883, as well as the species of crustaceans, *Cyclocyris laevis* (Müller, 1776) and *Cypria turneri* Hoff, 1942 (Petrochenko, 1956; Kakacheva-Avramova, 1983; Bauer (Ed.), 1987; Moravec, 2013). The established species of helminths are characterized by complex development cycles. *N. rutili* (Acanthocephala) and *Sch. petruschewskii* (Nematoda) are core species for the parasite communities of the barbel.

**Component community**

N. *rutile* (Acanthocephala) and *Sch. petruschewskii* (Nematoda) were distinguished with the highest prevalences (70% and 40%, respectively), followed by those of *C. laticeps* (27%) (Cestoda) and *All. isoporum* (Trematoda) (24%). The species *C. laticeps* and *All. isoporum* are component species of the host's parasite communities (Table 1).

*B. rectangulum* is an accidental species to the parasite communities of the barbel. The highest mean intensity is *N. rutili* (3.14), and the lowest is *B. rectangulum* (1.0) (Table 1). Ecological intensity and prevalence for *N. rutili* have the highest values and for *B. rectangulum* they are the lowest. Populations of *Sch. petruschewskii* in the barbel have a lower ecological intensity but a higher prevalence. In the remaining two populations, of *All. isoporum* and *C. laticeps*, almost the same ecological intensity and prevalence were observed. All identified parasite species are autogenous to the parasite communities of the barbel. The number and mean intensity of parasite species are closely related to the distribution, number, and intensity of intermediate and final hosts in the river ecosystem of the Cherna River.

Table1. Biodiversity, mean intensity (MI), and prevalence (P%) of parasite species of *Barbus cyclolepis* Heckel, 1837

| Parasite species | Intermediate hosts | Definitive host *B. cyclolepis* (N[1]= 30) | |
|---|---|---|---|
| | | P%[2] | MI[3] |
| Trematoda | | | |
| 1. *Allocreadium isoporum* (Loos, 1894) | Mollusca, I; Insecta, larvae, II | 24% | 1.4 |
| Cestoda | | | |
| 2.*Bathybothrium rectangulum* (Bloch, 1782) | Crustacea | 7.0% | 1.0 |
| 3.*Caryophyllaeus laticeps* (Pallas, 1781) | Oligochaeta | 27% | 1.6 |
| Nematoda | | | |
| 4.*Schulmanela petruschewskii* (Schulman, 1948) Ivashkin, 1964 | Oligochaeta | 40% | 1.5 |
| Acanthocephala | | | |
| 5.*Neoechinorhynchus rutili*(Müller, 1780) | Insecta Crustacea | 74% | 3.14 |

Legend: [1]N = total number of examined fish specimens.
[2]P% = prevalence.
[3]MI = mean intensity.

*B. rectangulum* is an accidental species to the parasite communities of the barbel. The highest mean intensity is *N. rutili* (3.14), and the lowest is *B. rectangulum* (1.0) (Table 1). Ecological intensity and prevalence for *N. rutili* have the highest values and for *B. rectangulum* they are the lowest. Populations of *Sch. petruschewskii* in the barbel have a lower ecological intensity but a higher prevalence. In the remaining two populations, of *All. isoporum* and *C. laticeps*, almost the same ecological intensity and prevalence were observed. All identified parasite species are autogenous to the parasite communities of the barbel. The number and mean intensity of parasite species are closely related to the distribution, number, and intensity of intermediate and final hosts in the river ecosystem of the Cherna River.

**Infracommunity**

Out of the 30 specimens of barbel examined, no parasites were found in only two specimens of fish (6.67%). The largest share of barbels infested with two types of helminths (50%), followed by those infested with one helminth (30%) and three types of helminths (13.34%). Brillouin's index of diversity is HB = 0.64 (Table 2).

Table 2. Infracommunity data

| Number of helminth species | | | | |
|---|---|---|---|---|
| Total number | 5 | | | |
| Number of infected fish | 0 | 1 | 2 | 3 |
| Number of helminth species | 2 | 9 | 15 | 4 |
| Number of helminth specimens | | | | |
| Total number | 51 | | | |
| Mean ± SD | 10.2±7.49 | | | |
| Range | 1-5 | | | |
| Mean HB ± SD | 0.64±0.21 | | | |

## DISCUSSIONS

The helminth species of *B. cyclolepis* found in this study have also been reported for other localities in Bulgaria (Table 3).

Table 3. Endohelminth species of *Barbus cyclolepis* reportedfrom other studies in Bulgaria

| Parasite species | Authors | Locality - rivers |
|---|---|---|
| Trematoda | | |
| *Allocreadium isoporum* (Loos, 1894) | Kakacheva-Avramova, 1965 | Syuyutlijka, Asenitsa |
| | Margaritov, 1965 | Vycha |
| Parasite species | Authors | Locality - rivers |
| | Kirin, 2002 | Luda Yana |
| | Kirin et al., 2020 | Tamrashka |
| Cestoda | | |
| *Caryophyllaeus brachycollis* Janiszewska, 1951 | Kakacheva-Avramova, 1965 | Asenitsa, Sushitsa |
| | Margaritov, 1965 | Maritsa, Vycha, Topolnitsa |
| *Caryophyllaeides fennica* (Schneider, 1902) Nybelin, 1922 | Kakacheva-Avramova, 1965 | Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijka, Sushitsa, Bedechka |
| | Margaritov, 1965 | Topolnitsa |
| | Kirin et al., 2020 | Tamrashka |
| *Caryophyllaeides* sp. | Kakacheva-Avramova, 1965 | |
| *Bathybothrium rectangulum* (Bloch, 1782) | Kakacheva-Avramova, 1965 | Asenitsa, Syuyutlijka |
| | Margaritov, 1965 | Maritsa, Vacha, Chepinska |
| | Kirin, 2002 | Luda Yana |
| | Kirin, 2003 | Arda |
| Cestoidea g. sp. | Margaritov, 1965 | Vycha |
| Acanthocephala | | |
| *Acanthocephalus anguillae* (Müller, 1780) | Margaritov, 1965 | Chepinska |
| *Neoechinorhynchus rutili* (Müller, 1780) | Kakacheva-Avramova, 1965 | Syuyutlijka |
| | Kakacheva-Avramova, 1972 | Tundzha |
| | Chunchukova et al., 2020 | Topolnitsa |
| *Pomphorhynchus laevis* (Zoega in Müller, 1776) | Chunchukova, 2020 | Chepelarska |
| | Kirin et al., 2020 | Tamrashka |
| Nematoda | | |
| *Rhabdochona denudata* (Dujardin, 1845) Raillet, 1916 | Kakacheva-Avramova, 1965 | inТракия |
| | Margaritov, 1965 | Maritsa, Vycha, Chepinska, Topolnitsa |
| *Rhabdochona hellichi* (Šramek, 1901) Chitwood, 1933 | Chunchukova, 2020 | Chepelarska |
| | Kirin et al., 2020 | Tamrashka |
| *Rhabdochona gnedini* Skrjabin, 1948 (syn., *Rhabdochona sulaki* Saidov, 1953) | Matgaritov, 1964 | Tundzha, Vycha |
| | Margaritov, 1965 | Maritsa, Vycha |
| | Kirin et al., 2020 | Tamrashka |
| *Capillaria* sp. | Margaritov, 1965 | Maritsa |
| *Schulmanella*sp. | Kakacheva-Avramova, 1972 | Tundzha |
| *Rhabdochona* sp. juv. | Kakacheva-Avramova, 1965 | Maritsa, Asenitsa |
| Nematoda d. sp. | Margaritov, 1965 | Topolnitsa |

Sixteen taxa have been reported from previous studies of *B. cyclolepis*. Two species are reported for the first time for the barbel helminth communities, *C. laticeps* and *Sch. petruschewskii* in this study. From the total of 18 taxa, 5 species were identified for barbel from the Cherna River (27.78%).

*Schulmanella* sp. was reported as a helminth species of *round-scaled barbel* from the Tundzha River, Bulgaria (Kakacheva-Avramova, 1972). Helminth species found in *B. cyclolepis* have also been reported for other fish species in Bulgaria (Kakacheva-Avramova, 1983; Chunchukova et al., 2020a, b; Zaharieva & Zaharieva, 2020a, b; Zaharieva & Zaharieva, 2020c, d; Zaharieva & Zaharieva, 2021a; Zaharieva & Zaharieva, 2021b).

## CONCLUSIONS

As a result of the examination of 30 specimens of *B. cyclolepis* from the Cherna River, infection with 5 types of endohelminths was found. Of these, two species are reported for the first time for the barbel fauna in Bulgaria, *C. laticeps* and *Sch. petruschewskii*. *C. laticeps* is a component species (P%=27) and *Sch. petruschewskii* (P%=40) is core species for the helminth communities of the barbell from the studied freshwater ecosystem. The five parasite species are autogenous to the parasite communities of the barbel.

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