Scientific Papers. Series D. Animal Science. Vol. LXIII, No. 2, 2020
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

NEW DATA ON THE HELMINTH FAUNA OF *ABRAMIS BRAMA* FROM THE DANUBE RIVER, BULGARIA

Mariya CHUNCHUKOVA, Diana KIRIN

Agricultural University-Plovdiv, Department of Agroecology and Environmental Protection, Mendeleev 12, Plovdiv, 4000, Bulgaria

Corresponding author email: m.chunchukova@abv.bg

Abstract

During the summer of 2017, 10 specimens of freshwater bream (Abramis brama (Linnaeus, 1758)) from the Bulgarian part of the Lower Danube River were examined with standard techniques for parasites. Helminth parasites were recorded in 9 freshwater bream specimens (90.00%) from the Danube River. Five species of parasites were identified: one cestode species Caryophyllaeus laticeps (Pallas, 1781), two trematode species from family Monorchiidae (Asymphylodora imitans (Mühling, 1898); Palaeorchis incognitus (Szidat, 1943)), one acanthocephalan Pomphorhynchus laevis (Zoega in Müller, 1776) and one nematode species Rhabdochona denudata (Dujardin, 1845). All established helminth species are autogenic species, matured in fish. In the component community of Abramis brama from Danube River P. laevis, P. incognitus and R. denudata are core species. A. imitans and C. laticeps are component parasite species for the helminth communities of A. brama. The established in this study parasite species are discussed and compared with previous researches of parasite communities of A. brama from River Danube in Bulgaria. As a result of this study is presented new data for helminths and helminth communities of A. brama.

Key words*:Abramis brama, Bulgaria, Danube,helminth communities, helminths.*

INTRODUCTION

River Danube is the Europe's second-longest river. The River is a habitat to many fish species and has an important place in the European ecological network. The shoreline zone of the Danube River in Bulgaria was presented by 44 fish species (Polačik et al., 2008). Fish parasites communities of *A.brama* from the Bulgarian part of River Danube were studied relatively often during the past few years (Margaritov, 1959; Kakacheva-Avramova, 1977; Atanasov, 2012; Kirin et al., 2013; Kirin et al., 2014; Chunchukova et al., 2016; Chunchukova et al., 2017). Freshwater bream can serve as a final and intermediate host to a wide range of parasite species (see Moravec, 2001). Parasites with their complex life cycles can be used to determine the food web structure and are useful bioindicators of ecosystem stability and biodiversity (Marcogliese and Cone, 1997; Marcogliese, 2003; Marcogliese, 2004).

This study aims to present the diversity and communities of parasites of freshwater bream from the Bulgarian part of the Lower Danube River (town of Silistra). As a result of this survey, new data for helminths and helminth communities of *A. brama* is presented.

MATERIALS AND METHODS

In the summer of 2017 fish and fish parasites were collected and examined from the Danube River (town of Silistra, Bulgarian part). The town of Silistra (44^0117'N, 27^0267'E) is situated on the riverside, in the north eastern part of the Danube Valley, on the last Bulgarian part of the Danube River.

A total of 10 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) from the Danube River were collected and examined in 2017. The fish were caught by angling. The scientific and common names of fish hosts are used according to the FishBase database (Fröse and Pauly, 2020).

The fish samples were examined immediately after their capture for gastrointestinal parasites using standard techniques. Trematodes were fixed as permanent slides after their colouring with acetic carmine, differentiation in 70% acid ethanol, dehydrating in increasing ethanol

series, clarifying in eugenol and mounting in Canada balsam (Bykhovskaya-Pavlovskaya, 1985; Georgiev et al., 1986). The samples were counted and identified using keys of Bauer et al. (1981), Bauer (1987) and Bykhovskaya-Pavlovskaya (1985). Cestodes were stained with acetic carmine and mounted as permanent slides in a Canada balsam, according to Georgiev et al. (1986) and Scholz and Hanzelová (1998). Acanthocephalans were examined as temporary slides in ethanol-glycerin and identified (Petrochenko, 1956; Ergens and Lom, 1970; Bykhovskaya-Pavlovskaya, 1985). Nematodes were examined as temporary microscopic preparations in glycerin (Moravec, 1994; 2013). The dominant structure of the component helminth communities was determined according to the criteria proposed by Kennedy (1993) based on the prevalence (P%): accidental (P% < 10), component (P% < 20) and core (P% >20) species. The ecological terms prevalence, mean intensity (MI) and mean abundance (MA) were used and calculated, based on Bush et al. (1997). Analyses of helminth community structure were carried out in both levels: infracommunity and component community. The component data is used to determine the total number of species, Shannon diversity index (H'), Pielou evenness index (E), Berger-Parker dominance index (d) according to Magurran (2004). The infracommunity data is used to calculate the mean number of species, the mean number of helminth specimens, Brillouin diversity index (HB) (Kennedy, 1993, 1997; Magurran, 2004).

RESULTS AND DISCUSSIONS

A total of 10 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) are collected and examined from the Danube River. The freshwater bream is not included in the Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011). *Abramis brama* is estimated as least concern species (LC=Least Concern; IUCN Red List Status). Freshwater bream is freshwater, brackish, benthopelagic, potamodromous fish species. Adults of this fish species inhabit a wide variety of lakes and large to medium-sized rivers. The diet of freshwater bream includes insects, particularly chironomids, small crustaceans, molluscs and plants. Larger specimens of *A. brama* may feed on small fish. Larvae and juveniles of freshwater bream live in still water bodies, feeding on plankton (Fröse and Pauly, 2020).

A total of 10 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) are collected and examined from the Danube River. Helminth parasites are recorded in 9 freshwater bream specimens (90.00%) from the Danube River. Five species of parasites are identified: one cestode species (*Caryophyllaeus laticeps* (Pallas, 1781)), two trematode species from family Monorchiidae (*Asymphylodora imitans* (Mühling, 1898); *Palaeorchis incognitus* (Szidat, 1943)), one acanthocephalan (*Pomphorhynchus laevis* (Zoega in Müller, 1776)) and one nematode species (*Rhabdochona denudata* (Dujardin, 1845)) (Table 1). All helminth species occurred as adults. They are all autogenic species, matured in fish.

Table 1. Species diversity of helminth parasites of *Abramis brama* from Danube River (N – number of examined fish specimens, n – number of infected hosts, p – number of parasites, P% – prevalence, MA – mean abundance, MI – mean intensity)

Helminth species	N=10					
	n	p	P%	MA±SD	MI±SD	Range
Caryophyllaeus laticeps (Pallas, 1781)	1	4	10.00	0.4±1.2	4.0±0	4
Asymphylodora imitans (Mühling, 1898)	2	13	20.00	1.3±2.69	4.33±3.30	2-9
Palaeorchis incognitus (Szidat, 1943)	3	8	30.00	0.8±1.78	2.67±2.36	1-6
Pomphorhynchus laevis (Zoega in Müller, 1776).	4	32	40.00	3.2±5.79	8.0±6.75	1-17
Rhabdochana denudata (Dujardin, 1845)	3	9	30.00	0.9±2.07	3.0±2.83	1-7

Component community

In the component community of *Abramis brama* from Danube River *P. laevis* (P%=40.00), *P. incognitus* (P%=30.00) and *R. denudata* (P%=30.00) are core species. *A. imitans* (P%=20.00) and *C. laticeps* (P%=10.00) are component parasite species for the helminth communities of *A. brama* (Table 1). In the component community of freshwater bream from Danube River acanthocephalans are presented with the highest number of specimens, with one species and 32 specimens. Trematodes are presented with two species and 21 specimens. Nematodes are represented by one species and nine specimens. Cestodes are represented by one species and four specimens.

In general, the parasite communities of *A. brama* are represented by five species of parasites belonging to four classes, four orders and four families. The total number of isolated and studied specimens is 66. The obtained results are related to Shannon diversity index H'= 1.368, Pielou evenness index E = 0.850 and Berger-Parker Dominance Index d=0.485 (Table 2).

Table 2. Basic indices of helminth community of *Abramis brama* from Danube River

Number of helminth species	5
Number of helminth specimens	66
H' (Shannon, diversity)	1.368
E (Pielou, evenness)	0.850
d (Berger-Parker Dominance Index)	0.485
Dominant species	*P. laevis*

Infracommunity

Species richness in infracommunity of freshwater bream ranges from 1 to 3 species. With one helminth species were infected six fishes (60.00 %), with two helminth species - 2 fishes (20.00%) and with three species - only one specimen of fish (10.00%). The largest number of helminth specimens established in a single host specimen is 24. The average species richness (mean number of species for fish specimen) in infracommunity of freshwater bream is 1.4±0.8 species (Table 3). The parasite communities of *A. brama* from the Danube River showed Brillouin's diversity index, HB=0.453± 0.162 (Table 3).

Caryophyllaeus laticeps is a parasite of fish from Cyprinidae family, but for more specific hosts are considered the species of the genus *Abramis* (Kakacheva-Avramova, 1983).

Table 3. Parameters of the infracommunities of *Abramis brama* from Danube River

Abramis brama	Number of endohelminth species				
	0	1	2	3	Mean±SD Range
	1	5	3	1	1.4±0.8 0-3
Abramis brama	Number of endohelminthspecimens				
	Total number		Brillouin's index HB		Mean± SD Range
	66		0.453±0.162		6.7±6.9 31-24

It was found in *Vimba vimba carinata*, *Abramis brama* and *Abrami sapa* from Bulgarian section of River Danube (Kakacheva-Avramova, 1977). The development of *C. laticeps* is done through an intermediate host – *Tubifex tubifex, Psammoryctides barbatus* and *Limnodrilus claparedianus* (Kakacheva-Avramova, 1983).

Asymphylodora imitans was found in *Abramis brama* and *Blicca bjoerkna* from the Bulgarian section of the Danube River (Kakacheva-Avramova, 1977; Chunchukova et al., 2017). According to Zhytova and Kornyushyn (2017) *Contectiana contecta* (Millet, 1813) =*Viviparus contectus* (Millet, 1813) is the main host of *A. imitans* in Ukrainian polissya waters, while *Planorbis planorbis* (Linnaeus, 1758) is an accidental host. Akimova (2015) reported 4 Gastropoda species from the water bodies of Belarus as intermediate hosts for *A. imitans* (*Radix balthica* (Linnaeus, 1758), *Radix ampla* (W. Hartmann, 1821), *Stagnicola corvus* (Gmelin, 1791) and *Radix auricularia* (Linnaeus, 1758) from the water bodies of Belarus.

*Palaeorchis incognitus*was reported for Danube River basin from barbel (Moravec et al., 1997). *P. incognitus* was reported from *A. brama* for Danube River and Latorica River (see Moravec, 2001), but so far has not been reported for Bulgaria. According to Akimova (2015) the gastropod *Bithynia tentaculata* is involved in the digenean life cycle as the first intermediate host by the 20 or more species of parasites, one of which is *P.incognitus*.

Pomphorhynchus laevis is an intestinal parasite of many freshwater fish, most often by a family Cyprinidae and less frequently by families Salmonidae, Percidae, Siluridae and others (Kakacheva-Avramova, 1983). *P. laevis* was

found in *Abramis brama* and other fish hosts from the Bulgarian section of the Danube River (Atanasov, 2012). This acanthocephalan develops with the participation of an intermediate host – *Gamarus pulex* (Amphipoda) (Petrochenko, 1956).

P. laevis was found in *A. brama* from Danube River (Kakacheva-Avramova, 1977; Atanasov, 2012). The intermediate host of *P. laevis* is *Gammarus pulex*, and definitive hosts are fish (Kakacheva-Avramova, 1983).

Rhabdochona denudata is an intestinal parasite of many species of Cyprinidae family (Moravec, 2013). As parasite of freshwater bream *R. denudata* was reported for Danube River basin (see Moravec, 2001). It is the first report of *R. denudata* as a parasite of *Abramis brama* from the Danube River, Bulgaria. Moravec (2013) suggested that in addition to mayflies also some other aquatic arthropods may serve as intermediate hosts of *R. denudata.*

For the Bulgarian section of River Danube, 29 species were reported as parasites of parasite communities of *A. brama* (Table. 4)

Table 4. Overview of helminth species of *Abramis brama* registered in the lower section of the Danube River, Bulgaria

Authority / Helminth species	Margaritov (1959)	Kakacheva-Avramova (1977)	Atanasov (2012)	Kirin et al. (2013)	Kirin et al. (2014)	Chunchukova et al. (2016)	Chunchukova et al. (2017)	This study
Cestoda								
Caryophyllaeus laticeps		•						•
Caryophyllaeides fennica	•	•						
Caryophyllaeus fimbriceps			•			•		
Trematoda								
Nicolla skrjabini		•						
Asymphylodora imitans							•	•
Asymphylodora tincae						•		
Palaeorchis incognitus								•
Dactylogyrus auriculatus	•							
Dactylogyrus distinguendus		•						
Dactylogyrus sphyrna		•						
Dactylogyrus zandti	•	•						
Gyrodactylus elegans	•			•	•			
Diplostomum spathaceum larv.	•		•					
Diplostomum pseudospathaceum larv			•					
Paradiplozoon homoion						•		
Dactylogyrus yinwenyingae						•		
Diplozoon paradoxum		•		•	•			
Metagonimus yokogawai			•					
Nematoda								
Raphidascaris acus larv.							•	
Rhabdochona denudata								•
Contracaecum microcephalum larv.						•		
Acanthocephala								
Acanthocephalus lucii						•	•	
Acanthocephalus anguillae						•	•	
Metechinorhynchus salmonis		•						
Pomphorhynchus laevis		•	•					•
Pomphorhynchus tereticollis				•	•		•	

In this study, the fixed five species of helminths present only 17.24% of those established for the freshwater bream from the Danube River in the Bulgarian section. In this study of helminth communities of freshwater bream from the Bulgarian part of River Danube, the prevalence (P%) was 90.00%. It is close to the established prevalence from Atanasov (2012) - 85.37% but differs from some more recent studies of *A. brama* from Bulgarian section of River Danube. Chunchukova et al. (2016) and Chunchukova et al. (2017) establish a lower prevalence - respectively 55.3% and 64.44%.

Summary data on Table 4 show that cestode species that refer *to A. brama* from Bulgarian part of Danube River all belong to order *Caryophyllidea.* The numbers of trematode species vary in different studies from one species (Chunchukova et al., 2017) to five species (Kakacheva-Avramova, 1977). It is due probably not only to various ecological factors in individual years but also to the fact that monogeneans were most probably rarely checked. The acanthocephalan *Pomphorhynchus laevis* is a euryxenous parasite having a wide host range, which includes at least 48 fish species (Moravec, 2001). For Bulgarian section of Danube River, this thorny-headed warm has been often reported from various fish host including freshwater bream (Margaritov, 1959, 1966; Kakacheva-Avramova, 1977; Nedeva et al., 2003; Nachev and Sures, 2009; Atanasov, 2012; Chunchukova et al., 2019; etc.). Nematode species were not referred in *A. brama* from Bulgarian part of Danube River in earlier investigations. Only in the most recent studies had been reported two nematode larvae, which also belong to order Rabditida, as the reported *Rhabdochana denudata* in this study (Table 4).

CONCLUSIONS

As a result of the investigation of 10 specimens of freshwater bream from the Danube River, five gastrointestinal parasite species were established: *Caryophyllaeus laticeps*, *Asymphylodora imitans*, *Palaeorchis incognitus*, *Pomphorhynchus laevis* and *Rhabdochona denudata*. The establishment of *P. incognitus* in *A. brama* represents a new host record for the Bulgarian section of River Danube. It is the first report of *R. denudata* as a parasite of *A.brama* from the Danube River, Bulgaria.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Agricultural University-Plovdiv, which provided the laboratory and technical equipment.

REFERENCES

Akimova, L. N. (2015). Significance of certain water gastropods in digenean circulation (Trematoda: Digenea) on the territory of Belarus. In: Conceptual and applied aspects of scientific research and education in the field of zoology of invertebrates: *Proceedings of the IV International Conference*, 146–154 (In Russian).

Atanasov, G. (2012). *Fauna, morphology and biology on the endohelminths of fish from Bulgarian part of the Danube River*. PhD these, Sofia (In Bulgarian).

Bauer, O. N., Musselius, V. A., Strelkov, Y. A. (1981). *Diseases of pond fish*. Moscow,RU: Legkaya Pishchevaya Promishlenost' Publishers (In Russian).

Bauer, O.N. (1987). *Key to the parasites of freshwater fishes in the fauna of the U.S.S.R*. Leningrad, RU: Academy of Sciences, USSR, Nauka.

Bush, A., Lafferty, K., Lotz, J., Shostak, A. (1997). Parasitology meets ecology on its own terms. *Journal of Parasitology*, 83, 575-583.

Bykhovskaya-Pavlovskaya, I. (1985). *Parasites of fish. Manual on study*, Leningrad, RU: Nauka, (In Russian).

Chunchukova, M., Kirin, D., Kuzmanova, D. (2019). Gastrointestinal helminth fauna and helminth communities of bleak (*Alburnus alburnus*, L. 1758) from lower section of Danube River, Bulgaria.*Bulgarian Journal of Veterinary Medicine*, 22 (3), 344–352.

Chunchukova, M., Kirin, D., Kuzmanova, D., Shukerova, S. (2017). Accumulation of lead in *Abramis brama* and its parasite *Pomphorhynchus tereticollis* from Danube river (Vetren area), Bulgaria. *Scientific Papers*. *Series D. Animal Science*, LX, 327-332.

Chunchukova, M., Shukerova, S., Kirin, D. (2016). Research of the impact of the River Danube on the Sreabarna Biosphere Reserve by the model ecosystem *Abramis brama* – Macroinvertebrates – Sediments. *Agricultural Sciences*, 19, 151-158.

Ergens, R., Lom, J. (1970). *Causative agents of fish diseases.* Prague, CZ:Academia, 384 (In Czech).

Fröse, R., Pauly D. (2020). FishBase. World Wide Web electronic publication, www.fishbase.org (02 February 2020, date last accessed).

Georgiev, B., Biserkov, V., Genov, T. (1986). In totostaining method for cestodes with iron acetocarmine. *Helminthologia*, 23, 279–281.

Golemanski, V. (Ed-in-Chief) (2011). *Red Data Book ofthe Republic of Bulgaria*. Sofia, BG: Joint edited of

the Bulg.Acad of Sci. and Ministry of Environment and Waters, Vol. 2. – Animalia (In Bulgarian).

*IUCN Red List Status, (n.d.)*www.iucnredlist.org

Kakacheva-Avramova, D. (1977). Studies on helminths of fishes in the Bulgarian section of the Danube River. *Helminthologia*, 3, 20-45.

Kakacheva-Avramova, D. (1983). *Helminths of freshwater fishes in Bulgaria*. Sofia, BG: Bulgarian Academy of Sciences (In Bulgarian).

Kennedy, C. (1993). The dynamics of intestinal helminth communities in eels *Anguilla anguilla* in a small stream: long-term changes in richness and structure. *Parasitology*, 107, 71-78.

Kennedy, C. (1997). Freshwater fish parasites and environmental quality, an overview and caution. *Parassitologia*, 39, 249-254.

Kirin, D., Hanzelova, V., Shukerova, S., Hristov, S., Turcekova, L., Spakulova, M. (2013). Helminth communities of fishes from the River Danube and Lake Srebarna, Bulgaria. *Scientific Papers. Series D. Animal Science*, LVI, 333-340.

Kirin, D., Hanzelová, V., Shukerova, S, Kuzmanova, D. (2014). Biodiversity, bioindication and helminth communities of *Abramis brama* (Linnaeus, 1758) from the Danube River and Srebarna Lake, Bulgaria. *Turkish Journal of Agricultural and Natural Sciences*, Special Issue 1, 727-733.

Magurran, A. E. (2004). *Measuring Biological Diversity*. Oxford, UK: Blackwell Publishing.

Marcogliese, D. J. (2004). Parasites: Small players with crucial roles in the ecological theatre. *Ecohealth*, 1, 151–64.

Marcogliese, D. J., Cone, D.K. (1997). Food webs: a plea for parasites. *Trends in Ecology & Evolution*, 12, 320–325.

Marcogliese, D. J. (2003). Food webs and biodiversity: are parasites the missing link? *Journal of Parasitology*, 89, S106–S113.

Margaritov, N. (1959). *Parasites of some freshwater fishes*. Varna, BG:Publishing House NIRRP, (In Bulgarian).

Moravec, F., Konecny, R., Baska, F., Rydlo, M., Scholz, T., Molnar, K., Schiemer, F. (1997). Enohelminth fauna of barbel, *Barbus barbus* (L.), under ecological conditions of the Danube basin in Central Europe. *Studie AV ČR*, 3, Academia, Praha.

Moravec, F. (2007). First experimental observations on the development of *Rhabdochona denudata* (Nematoda: Rhabdochonidae) in the intermediate host. *Folia Parasitologica*, 54, 236–238.

Moravec, F. (1994). *Parasitic nematodes of Freshwater Fishes of Europe*. Kluwer Academic Publishers, Dordrecht.

Moravec, F. (2001). *Checklist of the metazoan parasites of fishes of the Czech Republic and the Slovak Republic (1873–2000)*. Academia, Prague.

Moravec, F. (2013). *Parasitic Nematodes of Freshwater fishes of Europe*. Praha, CZ: Academia

Nachev, M., Sures, B. (2009). The endohelminth fauna of barbel (*Barbus barbus*) correlates with water quality of the Danube River in Bulgaria. *Parasitology*,136, 545–552.

Nedeva, I., Atanasov, G., Karaivanova, E., Cakis P., Lenhardt, M. (2003). *Pomphorhynchus laevis* (Müller, 1776) from the river Danube. *Experimental Pathology and Parasitology*, 6(13), 14-17.

Petrochenko, V.I. (1956). *Acanthocephala of Domestic and Wild Animals.* Moskow, RU: NAS of SSSR (In Russian).

Polačik, M., Trichkova, T., Janáč, M., Vassilev, M., Jurajda, P. (2008). The Ichthyofauna of the Shoreline Zone in the Longitudinal Profile of the Danube River, Bulgaria. *Acta Zoologica Bulgarica*, 60 (1), 77-88.

Scholz, T., Hanzelová, V. (1998). *Tapeworms of the genus Proteocephalus Weinland, 1858 (Cestoda: Proteocephalidae), parasites of fishes in Europe*. Praha, Academia.

Zhytova, E. P., Kornyushyn, V. V. (2017). The role of different mollusc species in maintaining the transmission of polyhostal trematode species in Ukrainian policy waters: the specificity of trematode parthenogenetic generations to mollusc hosts. *Vestnik zoologii*, 51(4), 295–310.