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Ecological study on helminths of three species of Gobiidae from the Danube River, Bulgaria

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Summary

A total of 72 specimens of *Babka gymnotrachelus* (Kessler, 1857), *Neogobius fluviatilis* (Pallas, 1814), and *Neogobius melanostomus* (Pallas, 1814) from four sampling sites along the Bulgarian section of the Danube River (Kudelin, Novo selo, Koshava, and Kutovo), Northwestern Bulgaria were submitted to ecologohelminthological investigation. During the examination 6 species of helminths have been identified from 3 classes: Trematoda (*Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960), Acanthocephala (*Acanthocephalus anguillae* (Müller, 1780) Lühe, 1911; *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911; *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908) and Nematoda (*Contracaecum* sp., *Eustrongylides excisus* Jägerskiöld, 1909). Ecological indices of the established endohelminth species were tracked. The four sampling sites from the Danube River are new habitats for the discovered endohelminth species of racer goby, monkey goby, and round goby. The three goby species are new host records: *B. gymnotrachelus* and *N. fluviatilis* for *Ac. lucii*; *N. melanostomus* for *Ac. lucii*, *Ac. anguillae* and *Contracaecum* sp. New species of helminths are found in the helminth fauna of the three studied species of gobies from the Danube River and the river basin (*Ac. lucii* of *N. fluviatilis*) and in Bulgaria (*Ac. lucii* of *B. gymnotrachelus*; *Ac. lucii*, *Ac. anguillae* and *Contracaecum* sp. of *N. melanostomus*). The pathogenic species helminths for the fish and humans are found.

Keywords: *Babka gymnotrachelus*; *Neogobius fluviatilis*; *Neogobius melanostomus*; Bulgarian section; ecological indices; endoparasitic species

Introduction

The Danube River crosses ten countries and flows into the Black Sea through a vast delta. The ichthyofauna of the river includes over 100 species, and 68 have been reported for the ichthyofauna of the Bulgarian section of the Danube (Zarev *et al*., 2013). For the section of the Danube River between Bulgaria and Romania, six species of gobies have been reported, including racer goby, *Babka gymnotrachelus* (Kessler, 1857; syn. *Neogobius gymnotrachelus*); monkey goby, *Neogobius fluviatilis* (Pallas, 1814); round goby, *Neogobius melanostomus* (Pallas, 1814; Vassilev *et al*., 2008). Four species of gobies, including *B. gymnotrachelus*, *N. fluviatilis*, and *N. melanostomus*, are spread upstream of the Danube River, outside their natural habitats (Ondračková *et al*., 2009). The Danube River plays an important role in expanding the range of various fish species (Bódis *et al*., 2012), which can lead to the spread of their parasites (Juhásová *et al*., 2019). Some non-native fish species become food for many native predatory fish species

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Fig. 1. Sampling sites, Danube River (https://www.google.bg/maps/place/Видин – https://www.google.bg/maps/place/Vidin)

(Bódis *et al.*, 2012). The infection of the fish is carried out through feeding (Docan *et al.*, 2019). The parasites' life cycles involve several intermediate hosts (Kuzmanova *et al.*, 2019), including fish. Fish are rich in nutrients (Ljubojevic *et al.*, 2015) and are often present in the human diet (Bănăduc *et al.*, 2016). A person can become infected by consuming raw fish or fish without sufficient heat treatment (Scholz, 1999; Ljubojevic *et al.*, 2015). Among the endoparasitic species causing zoonoses are *E. excisus* (Ljubojevic *et al.*, 2015; Juhásová *et al.*, 2019) and *Contracaecum* sp. (Demir & Karakişi, 2014; Shamsi, 2019). Different authors have studied the parasite fauna of gobies from the Danube River in other countries (Ondračková *et al.*, 2005; Molnár, 2006; Kosuthova *et al.*, 2009; Ondračková *et al.*, 2009; Mühlegger *et al.*, 2010; Francová *et al.*, 2011; Ondračková *et al.*, 2012; and others). The parasite fauna of *N. fluviatilis*, *N. melanostomus*, and others from the Black Sea was studied by Kvach (2005). Helminthological studies on gobies, including racer goby, monkey goby, and round goby, have also been carried out in the Danube River and the river basin in Bulgaria (Francová *et al.*, 2011; Ondračková *et al.*, 2010; Ondračková *et al.*, 2012; Kirin *et al.*, 2013; and others).

The present study aims to provide new data on the helminths and helminth communities of the three species of gobies (*B. gymnotrachelus*, *N. fluviatilis*, *N. melanostomus*) from the upper section of the Danube River (near the villages Kudelin, Novo selo, Koshava, and Kutovo) in Northwestern Bulgaria. The study enriches the data on the ecological indices of endohelminths of the three species of gobies. More research is needed on the parasite fauna of fish, especially those frequently consumed by humans and infested with pathogenic endohelminth species.

Material and Methods

In 2019 – 2021, during the spring, summer, and autumn, an ecological-helminthological study was conducted on a total of 72 specimens of gobies (Gobiidae) of the species: racer goby, *Babka gymnotrachelus* (Kessler, 1857); monkey goby, *Neogobius fluviatilis* (Pallas, 1814); round goby, *Neogobius melanostomus* (Pallas, 1814) near the villages Kudelin, Novo selo, Koshava and Kutovo, denoted as sampling sites (44°12'07.9"N 22°41'27.0"E; 44°09'52.4"N 22°47'09.8"E; 44°03'32.3"N 23°02'02.8"E and 44°00'58.4"N 22°58'30.3"E), the Danube River, Northwestern Bulgaria (Fig. 1). *B. gymnotrachelus*, *N. fluviatilis* are freshwater, brackish, and benthopelagic species. *N. melanostomus* is a marine, freshwater, brackish and benthic species. They are found in the Danube River,

Table 1. Metric data (L – total length, H – maximum height, W – body weight) of the studied specimens of *Babka gymnotrachelus*, *Neogobius fluviatilis*, *Neogobius melanostomus*.

The Danube River		L (cm)	H (cm)	W (g)
***Babka gymnotrachelus* N = 9**	**min – max**	8.8 – 16	1.2 – 2.5	4 – 37
	Mean ± SD	10.83 ± 2.24	1.73 ± 0.46	12.78 ± 10.60
***Neogobius fluviatilis* N = 48**	**min – max**	5.4 – 12	0.8 – 2.4	1 – 18
	Mean ± SD	8.61 ± 1.39	1.39 ± 0.31	4.49 ± 3.23
***Neogobius melanostomus* N = 15**	**min – max**	6.3 – 11.6	1.1 – 2.4	2 – 13
	Mean ± SD	8.23 ± 1.66	1.56 ± 0.34	6.07 ± 3.49

the lower current and mouths of its tributaries, in the mouths of rivers flowing into the Black Sea and others. The three species feed on crustaceans, insects, and others. *B. gymnotrachelus* inhabits muddy, sandy, or stony bottoms; *N. fluviatilis* prefers muddy and sandy bottoms; *N. melanostomus* is found on stony and sandy bottoms (Karapetkova & Zhivkov, 2006; Vassilev *et al*., 2012). The three studied species of gobies are in the category "LC = Least Concern" on the IUCN Red List. The monkey goby is also included in Annex No. 3 of the Bern Convention (Freyhof & Brooks, 2011; https://www.iucnredlist.org).

Collecting fish samples was carried out under the permit for fishing for scientific research purposes from the Executive Agency for Fisheries and Aquaculture, Ministry of Agriculture. Nine specimens of *B. gymnotrachelus* (Kudelin and Novo selo), 48 specimens of *N. fluviatilis* (Kudelin, Koshava, Novo selo, and Kutovo) and 15 specimens of *N. melanostomus* (Kudelin, Koshava, and Novo selo) were subjected to ecologohelminthological examination.

The scientific names of the fish species are used according to Vassilev *et al.* (2012); Karapetkova and Zhivkov (2006). Immediately after their capture, all fish were weighed and measured; the metric data – total length, L, cm; maximum height, H, cm; body weight, W, g, were recorded (Table 1).

Fish were euthanized by being in a container with a drop of oil of cloves. A visual inspection of the fish's body's surface was performed, followed by dissection and fixation of the internal organs in ethyl alcohol for further processing. All caught specimens of gobies were examined for the presence of parasites according to standard methods (Zashev & Margaritov, 1966; Bauer (Ed), 1987; Moravec, 2013). The isolated parasites were fixed and preserved in 70 % ethyl alcohol until further processing. From the isolated helminths from class Trematoda, permanent microscope slides (by Georgiev *et al.*, 1986), and from the representatives of classes Acanthocephala and Nematoda – temporary microscope slides (by Zashev & Margaritov, 1966; Petrochenko, 1956; Moravec, 2013) were prepared. Basic ecological indices: total number of species; the total number of specimens; mean intensity (MI); mean abundance (MA); prevalence (P%), and range (R; min – max; by Bush *et al*., 1997) were presented.

Table 2. Distribution of established helminth species in *B. gymnotrachelus*, *N. fluviatilis*, and *N. melanostomus* from four biotopes located in the upper section of the Danube River in Bulgaria.

Fish species	***Babka gymnotrachelus*** **N = 9**		***Neogobius fluviatilis*** **N = 48**				***Neogobius melanostomus*** **N = 15**		
Sampling sites **Helminth species**	Kudelin N = 5	Novo selo N = 4	Kudelin N = 31	Koshava N = 5	Novo selo N = 11	Kutovo N = 1	Kudelin N = 8	Koshava N = 3	Novo selo N = 4
Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960	•	•	•		•	•	•	•	
Acanthocephalus anguillae (Müller, 1780) Lühe, 1911								•	
Acanthocephalus lucii (Müller, 1776) Lühe, 1911	•	•	•		•		•		•
Eustrongylides excisus Jägerskiöld, 1909	•	•	•		•				
Pomphorhynchus laevis (Zoega in Müller, 1776) Porta, 1908								•	
Contracaecum sp.							•		

Table 3. Species diversity and ecological indices in the helminth community of *Babka gymnotrachelus* from the Danube River (N – number of investigated fish; n – number of infected fish; p – number of fish helminths; MI – mean intensity; MA – mean abundance; P% – prevalence; R – range)

Babka gymnotrachelus **(N = 5 / Kudelin)** **Helminth species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Nicolla skrjabini	1	52	52.00	10.40	20.00	52
Acanthocephalus lucii	2	33	16.50	6.60	40.00	3-30
Eustrongylides excisus	2	3	1.50	0.60	40.00	1-2
Babka gymnotrachelus **(N = 4 / Novo selo)** **Helminth species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Nicolla skrjabini	3	292	97.33	73.00	75.00	15-237
Acanthocephalus lucii	3	59	19.67	14.75	75.00	2-39
Eustrongylides excisus	1	9	9.00	2.25	25.00	9

Ethical Approval and Informed Consent

This research on fish has complied with all the relevant national regulations and institutional policies for the care and use of animals.

Results

Ecologohelminthological research

For *B. gymnotrachelus* and *N. fluviatilis*, an infection with 3 helminth species and *N. melanostomus* with five endohelminth species was found. Two endohelminth species (*N. skrjabini* and *Ac. lucii*) were common to the three studied species of gobies. One endohelminth species (*E. excisus*) was common to *B. gymnotrachelus* and *N. fluviatilis*. Three endohelminth species (*Ac. anguillae*, *P. laevis*, *Contracaecum* sp.) were found only at *N. melanostomus* (Table 2).

Ecologohelminthological research of Babka gymnotrachelus

In *B. gymnotrachelus* from the two examined sampling sites (Kudelin and Novo selo), three identical endohelminth species were found. Of them, *N. skrjabini* and *Ac. lucii* had higher ecological indices in racer goby from Novo selo. *E. excisus* had higher mean intensity, mean abundance and range in racer goby from Novo selo but a higher prevalence in Kudelin (Table 3).

Ecologohelminthological research of Neogobius fluviatilis

As a result of the ecologohelminthological research of *N. fluviatilis* from four sampling sites (Kudelin, Novo selo, Kutovo, and Koshava), a high number of helminth species (3 species) was established in Kudelin and Novo selo. One helminth species was found in monkey goby from Kutovo. In the examined specimens, *N. fluviatilis* from Koshava helminths were not found. The trematode

Table 4. Species diversity and ecological indices in the helminth community of *Neogobius fluviatilis* from the Danube River (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)

Neogobius fluviatilis **(N = 31 / Kudelin)** **Helminth species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Nicolla skrjabini	15	47	3.13	1.52	48.39	1-17
Acanthocephalus lucii	10	55	5.50	1.77	32.26	1-38
Eustrongylides excisus	8	13	1.63	0.42	25.81	1-2
Neogobius fluviatilis **(N = 11 / Novo selo)** **Helminth species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Nicolla skrjabini	6	9	1.50	0.82	54.55	1-2
Acanthocephalus lucii	1	1	1.00	0.09	9.09	1
Eustrongylides excisus	1	1	1.00	0.09	9.09	1
Neogobius fluviatilis **(N = 1 / Kutovo)** **Helminth species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Nicolla skrjabini	1	3	3.00	3.00	100.00	3

Table 5. Species diversity and ecological indices in the helminth community of *Neogobius melanostomus* from the Danube River (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)

Neogobius melanostomus **(N = 8 / Kudelin)** **Helminth species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Nicolla skrjabini	5	16	3.20	2.00	62.50	1-8
Acanthocephalus lucii	5	123	24.60	15.38	62.50	1-90
Contracaecum sp.	1	1	1.00	0.13	12.50	1
Neogobius melanostomus **(N = 3 / Koshava)** **Helminth species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Nicolla skrjabini	1	1	1.00	0.33	33.33	1
Acanthocephalus anguillae	1	38	38.00	12.67	33.33	38
Pomphorhynchus laevis	1	11	11.00	3.67	33.33	11
Neogobius melanostomus **(N = 4 / Novo selo)** **Helminth species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Acanthocephalus lucii	1	1	1.00	0.25	25.00	1

N. skrjabini was a common parasite species of monkey goby from Kudelin, Novo selo, and Kutovo. The highest mean intensity and range had *N. skrjabini* from Kudelin, while the highest mean abundance and prevalence had *N. skrjabini* from Kutovo. *N. skrjabini*, *Ac. lucii* and *E. excisus* were common parasite species for monkey goby from Kudelin and Novo selo, as higher MI, MA, P%, and R were reported for the helminths from Kudelin. The exception is the prevalence of *N. skrjabini*, which was higher in monkey goby from Novo selo (Table 4).

Ecologohelminthological research of Neogobius melanostomus
At the examination of *N. melanostomus* for the presence of parasites from three sampling sites (Kudelin, Novo selo, and Koshava), a high number of helminth species (three species) was established in Kudelin and Koshava. In round goby from Novo selo, one helminth species was found. None of the helminths of *N. melanostomus* were found in the three sampling sites. *N. skrjabini* was a common round goby species from Kudelin, Koshava, and *Ac. lucii* – from Kudelin and Novo selo. The trematode *N. skrjabini* in round goby from Kudelin was distinguished by higher mean intensity, abundance, prevalence and range values than *N. skrjabini* from Koshava. The acanthocephalan *Ac. lucii* in round goby from Kudelin had higher mean intensity, mean abundance, prevalence, and range than *Ac. lucii* from Novo selo (Table 5).

Comparative examination of the ecological indices of the helminths common to the three species of gobies found in this study
Common helminth species for the three studied species of gobies from the upper section of the Danube River in Bulgaria (Kudelin, Koshava, Novo selo, Kutovo) were *N. skrjabini* and *Ac. lucii*. The trematode *N. skrjabini* had the highest mean intensity and mean abundance in racer goby from Novo selo and the highest prevalence – in monkey goby from Kutovo. *N. skrjabini* had the lowest values for MI and MA in round goby from Koshava and the lowest value for P% – in racer goby from Kudelin. *Ac. lucii* in round goby from Kudelin had the highest mean intensity and mean abundance and *Ac. lucii* in racer goby from Novo selo had the highest prevalence. The lowest and equal mean intensity was *Ac. lucii* in monkey goby and round goby from Novo selo, with the lowest mean abundance and prevalence, was *Ac. lucii* in monkey goby from Novo selo (Fig. 2).
N. skrjabini of *B. gymnotrachelus* from Novo selo had the highest range (R = 15 – 237), while *Ac. lucii* of *N. melanostomus* from Kudelin had the highest range (R = 1 – 90).

Discussion

During the ecologohelminthological research of the three species of gobies, 6 endohelminth species, of which 3 pathogenic species were found. *E. excisus* is a pathogenic species to fish (Novakov *et al*., 2015) and humans (Juhásová *et al*., 2019). *P. laevis* is a pathogenic fish species (Novakov *et al*., 2015). *Contracaecum* sp. is pathogenic to fish and humans (Zashev & Margaritov, 1966; Demir & Karakişi, 2014). In this study, *P. laevis* was found in the intestines of the examined fish; *E. excisus* is localized under the serous membrane on the surface of the mesentery and internal organs, in the body cavity, and the muscle under the skin; *Contracaecum* sp., larvae were found capsulated on the serous membrane of the organs in the abdominal cavity. *P. laevis* affects its hosts, damaging their intestines and slowing their growth. *E. excisus* damages the organs of fish (Novakov *et al*., 2015), and in humans, it causes intestinal perforation (Juhásová *et al*., 2019). *Contracaecum* sp.,

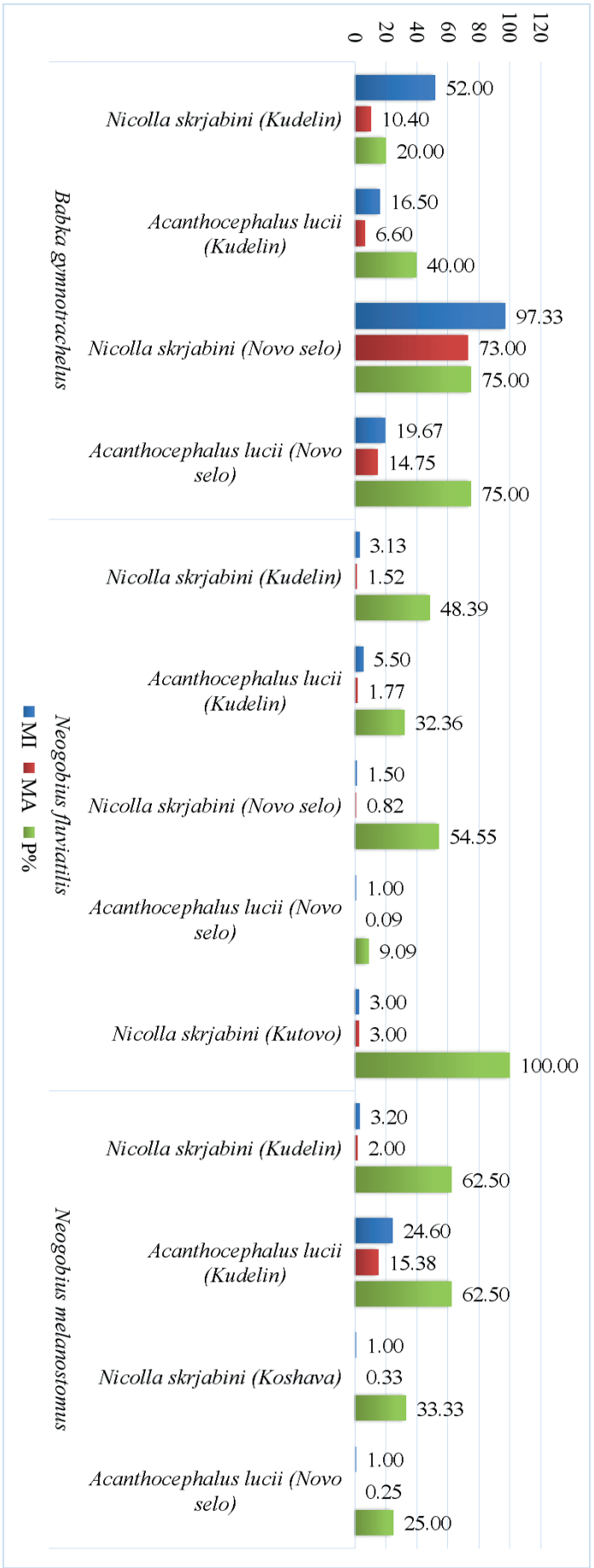

Fig. 2. Comparative examination of the ecological indices (MI, MA, and P%) of *Nicolla skrjabini* and *Acanthocephalus lucii* from the upper section of the Danube River in Bulgaria.

Table 6. Species composition of parasites of *Babka gymnotrachelus*, *Neogobius fluviatilis*, *Neogobius melanostomus* from the Danube River and its basin in different countries (* – endohelminths also found in the present study from the respective fish species)

Fish species	Authors	Sampling sites	Endohelminth species
Babka gymnotrachelus	Ondračková *et al*. (2012)	Danube River, Bulgaria – Vidin town, Koshava and Gomotartsi villages	**N. skrjabini* (MA = 3.41, P% = 84.4); *Raphidascaris acus* (Boch, 1779); **E. excisus* (MA = 0.41, P% = 28.1); *P. laevis*
Neogobius fluviatilis	Margaritov (1966)	Danube River, Bulgaria –between the mouth of the Timok River and Novo Selo village	**N. skrjabini*; *P. laevis*; *C. bidentatum*; *Rhabdochona* sp.; Acanthocephala gen sp.
	Kakacheva-Avramova (1977)	Danube River, Bulgaria	**N. skrjabini*; *P. laevis*; *C. bidentatum*
	Kakacheva-Avramova *et al*. (1978)	Danube River, Bulgaria – Vidin and Lom towns	**N. skrjabini*; *C. bidentatum*; *Contracaecum* sp. (larvae); *Rhabdochona* sp. (larvae); *P. laevis*
	Molnár & Székely (1995)	Lake Balaton, Hungary	*Ligula* sp. (larvae); *Proteocephalus* sp.; *Ang. crassus* (larvae)
	Ondračková *et al*. (2005)	Hron River, Slovakia	**N. skrjabini*; *P. laevis* (larvae); *Contracaecum* sp. (larvae); *R. acus* (larvae)
	Molnár (2006)	Danube River, Hungary	**N. skrjabini*; *P. laevis*; *R. acus* (larvae)
	Kosuthova *et al*. (2009)	Danube River, Slovakia	**N. skrjabini*; *P. laevis*
	Ondračková *et al*. (2012)	Danube River, Bulgaria – Koshava and Gomotartsi villages	**N. skrjabini* (MA = 8.76, P% = 52.6); **E. excisus* (MA = 0.32, P% = 15.8); *P. laevis*
	Kirin *et al*. (2013)	Danube River, Bulgaria – Vetren village	*Pomphorhynchus tereticollis* (Rudolphi, 1809) Meyer, 1932
Neogobius melanostomus	Ondračková *et al*. (2005)	Danube River, Slovakia	**N. skrjabini*; **P. laevis* (larvae); *R. acus* (larvae)
	Molnár (2006)	Danube River, Hungary	**N. skrjabini*; **P. laevis*
	Kosuthova *et al*. (2009)	Danube River, Slovakia	**P. laevis*
	Mühlegger *et al*. (2010)	Danube River, Austria	**N. skrjabini*; **Ac. lucii*

	Ondračková *et al*. (2010)	Danube River, Austria – Orth an der Donau	**P. laevis*; *R. acus*
		Danube River, Bulgaria – Vidin town	**P. laevis* (MA = 54.5, P% = 96.4; MA = 97.5, P% = 100); *R. acus*
	Francová *et al*. (2011)	Danube River, Austria – Orth an der Donau	**N. skrjabini*; **P. laevis*; *E. excisus* (larvae); *R. acus* (larvae)
		Danube River, Slovakia – Gabčíkovo	**P. laevis*; *R. acus* (larvae); *C. lacustris*
		Danube River, Bulgaria – Vidin and Ruse towns	**N. skrjabini* (MA = 0.1, P% = 3.6 for Vidin; MA = 0.3, P% = 7.9 for Ruse); *Nicolla* sp.; **P. laevis* (MA = 43.3, P% = 99.4 for Vidin; MA = 22.1, P%=94.7 for Ruse); *E. excisus*, larvae; *R. acus*, larvae
Neogobius melanostomus			
	Atanasov (2012)	Danube River, Bulgaria – Archar, Dobri Dol and Gomotartsi villages	*E. excisus*
	Ondračková *et al*. (2012)	Danube River, Austria – Orth an der Donau	**N. skrjabini*; *R. acus*; **P. laevis*
		Danube River, Bulgaria – Vidin town	**N. skrjabini* (MA = 0.11, P% = 5.3); *R. acus*; **P. laevis* (MA = 18.4, P% = 94.7); *E. excisus*
	Ondračková *et al*. (2021)	Morava River, the Czech Republic	*Eustrongylides* spp. (larvae)

larvae can reduce fish weight and liver lipid content and cause their death. In humans, they cause the disease anisakidosis (Zashev & Margaritov, 1966; Demir & Karakişi, 2014). *Eustrongylides* spp. cause gastritis and intestinal perforation in humans infected with this species (Ljubojevic *et al.*, 2015).

The ecological indices of helminths of the three fish species from the present study were compared with those from previous helminthological studies of the same fish species from the Bulgarian section of the Danube River. The helminths of *B. gymnotrachelus* from the Danube River in the area of Vidin town and the Koshava and Gomotartsi villages were studied by Ondračková *et al*. (2012). The authors reported 4 endohelminth species. Common helminth species with those established in the present study of racer goby were: *N. skrjabini* and *E. excisus*. The mean abundance of *N. skrjabini* from Kudelin and Novo selo was higher, while the prevalence was lower than those reported by Ondračková *et al*. (2012). The mean abundance and prevalence of *E. excisus* from Kudelin and the mean abundance of *E. excisus* from Novo selo were higher than those reported for Vidin, Koshava and Gomotartsi (Table 6). *B. gymnotrachelus* is a new host of *Ac. lucii* from the Danube River and the river basin in other countries and Bulgaria. *Ac. lucii* is reported for the first time for the helminth fauna of *B. gymnotrachelus* in Bulgaria. Kudelin and Novo selo are new habitats for *N. skrjabini*, *Ac. lucii* and *E. excisus* of *B. gymnotrachelus*.

More recent studies on the helminths of *N. fluviatilis* from the Bulgarian section of the Danube River have been carried out by Ondračková *et al*. (2012; in the area of the Koshava and Gomotartsi villages) and by Kirin *et al*. (2013; in the area of the Vetren village). Three helminth species were reported by Ondračková *et al*. (2012). Comparing the ecological indices (MA and P%) of *N. skrjabini* from Kudelin, Novo selo, and Kutovo with those indicated by Ondračková *et al*. (2012), it was found that generally, the ecological indices in the present study were lower, except the prevalence of *N. skrjabini* from Novo selo and Kutovo. The mean abundance and prevalence of *E. excisus* from Kudelin were higher, while those from Novo selo were lower than those indicated by Ondračková *et al*. (2012). The acanthocephalan *Pomphorhynchus tereticollis* (Rudolphi, 1809) Meyer, 1932, which was not found in

the present study in *N. fluviatilis*, was reported by Kirin *et al*. (2013; Table 6). *N. fluviatilis* is a new host of *Ac. lucii* from the Danube River and the river basin. *Ac. lucii* and others were reported in *N. fluviatilis* from the Black Sea (Kvach, 2005). *Ac. lucii* is reported for the first time for the helminth fauna of monkey goby from the Danube River and the river basin. Kudelin, Novo selo, and Kutovo are new habitats for the established helminths of *N. fluviatilis*.

Studies on the helminths of *N. melanostomus* from the Bulgarian section of the Danube River were carried out by Ondračková *et al*. (2010), Ondračková *et al*. (2012) in the area of Vidin town, and Francová *et al*. (2011) in the vicinities of the towns of Vidin and Ruse. Two, four and five helminth species were reported in round goby by Ondračková *et al*. (2010), Ondračková *et al*. (2012) and Francová *et al*. (2011), respectively. The mean abundance and prevalence of *N. skrjabini* and *P. laevis* in round goby from the present study were compared with those reported in earlier studies from the Bulgarian section of the Danube River. The MA and P% values of *N. skrjabini* from Kudelin and Koshava were higher than those reported by Francová *et al*. (2011) and by Ondračková *et al*. (2012). The mean abundance and prevalence of *P. laevis* in round goby from Koshava were lower than those indicated by Ondračková *et al*. (2010), Francová *et al*. (2011) and Ondračková *et al*. (2012) (Table 6). *N. melanostomus* is a new host of *Ac. lucii*, *Ac. anguillae* and *Contracaecum* sp. in Bulgaria. *Ac. lucii*, *Contracaecum rudolphii* Hartwich, 1964 L3, *Contracaecum microcephalum* (Rudolphi, 1809) Baylis, 1920 L3 and others were reported in *N. melanostomus* from the Black Sea (Kvach, 2005). The helminths *Ac. lucii*, *Ac. anguillae* and *Contracaecum* sp. are reported for the first time for the helminth fauna of *N. melanostomus* in Bulgaria. Kudelin, Koshava and Novo selo are new habitats for the established helminths of round goby.

Due to the place of localization of the helminths and the inclusion of the three species of gobies in the human diet, it is recommended to perform a visual inspection of the abdominal cavity and the muscles of the three species of gobies; to remove the internal organs, especially of the smaller specimens of fish and to cook to a sufficiently high internal temperature. Due to the presence of pathogenic helminth species, not only for fish but also for humans, it is desirable to conduct systematic studies on the parasite fauna of the three species of gobies, not only from the freshwater ecosystem of the Danube River but also from other aquatic ecosystems. It is important to carry out helminthological studies on predatory fish species that feed on the gobies and are consumed by humans. It is also desirable to monitor the ecological indices of the helminths.

Conflict of Interest

The authors state no conflict of interest.

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