Scientific Papers. Series D. Animal Science. Vol. LXVI, No. 1, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ECOLOGOHELMINTHOLOGICAL INVESTIGATION OF *Cobitis elongata*, *Cobitis taenia*, AND *Sabanejewia bulgarica* (Cobitidae) FROM THE DANUBE RIVER, BULGARIA

Petya ZAHARIEVA, Radoslava ZAHARIEVA, Diana KIRIN

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

Corresponding author email: petya.zaharieva3@gmail.com

Abstract

For the period 2019-2021, three fish species of the family Cobitidae, caught from the upper section of the Danube River in Bulgaria, were subjected to ecologohelminthological investigation. Four specimens of Cobitis elongata Heckel & Kner, 1858 (two specimens from Kudelin biotope and two from Koshava biotope); six specimens of spined loach (Cobitis taenia Linnaeus, 1758) (from Koshava biotope) and one specimen of Sabanejewia bulgarica Drensky, 1928 (from Kudelin biotope) are objects of research. The trematode Asymphylodora tincae (Modeer, 1790) Lühe, 1909 was reported for the first time as a helminth of S. bulgarica. The nematode Pseudocapillaria tomentosa (Dujardin, 1845) Moravec, 1987 was reported for the first time as a helminth of C. elongata. Kudelin and Koshava biotopes are new habitats for the found helminth species. During the helminthological examination of C. taenia, infection with helminths was not found. The study provides new data on helminth fauna and ecological indices (MI, MA, and P%) in the helminth communities of C. elongata and S. bulgarica.

Key words*: ecological indices, fish species, helminth species, Koshava, Kudelin.*

INTRODUCTION

The Danube River is the second longest river in Europe. The river is distinguished by an exceptional diversity of ichthyofauna (Juhásová et al., 2019). Studies on the parasite fauna of fish from the Danube River and the river basin in Bulgaria are carried out by various authors (Kirin et al., 2013; Kirin et al., 2014; Kuzmanova et al., 2019; Chunchukova & Kirin, 2020; Chunchukova et al., 2020; etc.). *Cobitis elongata*; *Cobitis taenia* and *Sabanejewia bulgarica* (syn. *Cobitis bulgarica* Drensky, 1928; *Cobitis aurata bulgarica* Drensky) are among the species poorly studied for parasites. Helminthological studies on *S. bulgarica* from the Bulgarian section of the Danube River are scarce (Margaritov, 1966; Kakacheva-Avramova, 1977; Kakacheva-Avramova et al., 1978). Studies on the parasite fauna of spined loach and *C. elongata* from the Bulgarian section of the river are lacking.

The present study aims to provide new data on the helminths of three fish species belonging to the Cobitidae family from the freshwater ecosystem of the Danube River in Bulgaria.

MATERIALS AND METHODS

For the period 2019-2021, 3 species of fish were collected - *Cobitis elongata* Heckel & Kner, 1858; spined loach, *Cobitis taenia* Linnaeus, 1758 and *Sabanejewia bulgarica* Drensky, 1928, caught from two places along the Danube River in the area of Kudelin and Koshava villages (marked as biotopes), Vidin Province, Northwestern Bulgaria. Kudelin biotope (44°12'07.9"N, 22°41'28.2"E) is located on the right bank of the Danube River, shortly after the river enters Bulgarian territory; about 35 km from the town of Vidin. Koshava biotope (44°03'59.9"N, 23°02'10.2"E) is also located on the right bank of the Danube River; about 20 km from the town of Vidin (Figures 1-2).

Fish were collected in accordance with the requirements of the Executive Agency for Fisheries and Aquaculture for catching fish for scientific research purposes. Fish species are represented by Vostradovsky (1973); Karapetkova & Zhivkov (2006); Kottelat & Freyhof (2007). In the field, immediately after capture, metric data - total length (TL) and

maximum height (MH) of the body in centimeters; body weight (BW) in grams was determined on each of the fish specimens (Table 1).

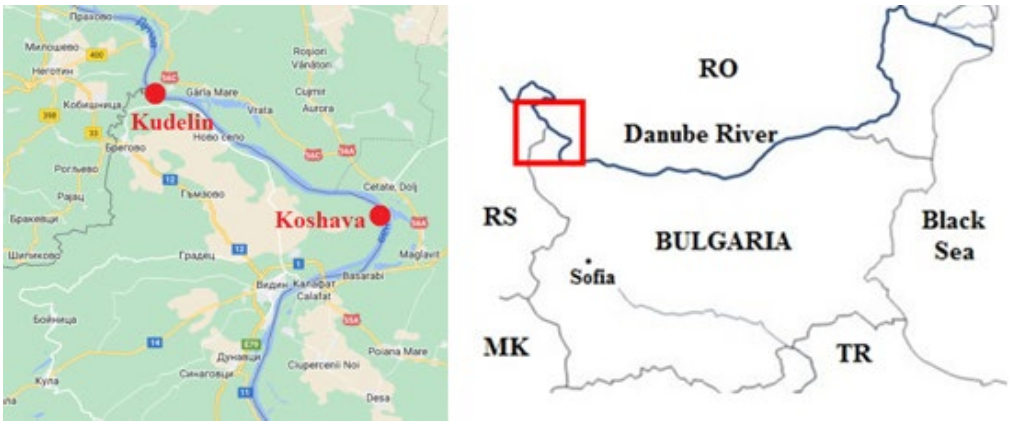

Figure 1. Location of Kudelin and Koshava biotopes along the Danube River, Vidin Province, Bulgaria (https://www.google.bg/maps/place/Видин)

Figure 2. Views from Danube River, Kudelin and Koshava biotopes; left to right (author's photos)

Table 1. Metric data (TL, MH, BW) of the examined specimens *Cobitis elongata*, *Cobitis taenia*

Danube River		TL (cm)	MH (cm)	BW (g)
***Cobitis elongata* N = 4**	**min-max**	5.3-8.9	0.6-1.3	1-2
	Mean±SD	7.58±1.57	0.95±0.29	1.50±0.58
***Cobitis taenia* N = 6**	**min-max**	6.5-9	1-1.4	1-4
	Mean±SD	8.02±0.90	1.23±0.20	2.83±1.33

A total of 11 specimens from the three fish species were examined by the method of the complete helminthological autopsy of the organs (Zashev & Margaritov, 1966; Moravec, 2013).

From the representatives of class Trematoda and class Nematoda, permanent and temporary microscopic preparations were prepared, respectively (Dubinina, 1948; Zashev & Margaritov, 1966; Moravec, 2013).

A microscope "XS-213" China was used to determine the type of helminths. The taxonomic affiliation of the isolated parasites was determined (by Bauer (Ed.), 1987; Moravec, 2013; and others).

RESULTS AND DISCUSSIONS

Fish species

Three freshwaters, demersal fish species, inhabiting the Danube River and its tributaries were studied. *C. elongata* prefers rivers with a moderate to fast current and a sandy bottom. The body is up to 17 cm long, and the weight - is up to 20 g. *C. taenia* inhabits slow-flowing and clean waters with a sandy bottom. The body

length of the species reaches up to 15 cm, and the weight - is up to 15 g. The lifespan is 3-4 years. Spawns in the spring. It has a slow growth rate. *S. bulgarica* prefers rivers with fast currents and gravel bottoms. The species has a body length of up to 10 cm and a weight of up to 25 g. It spawns in the spring, entering the mouths of some of the Danube tributaries (Ogosta, Iskar, Vit, Osam, and Yantra rivers) to breed. The diet of the three studied fish species consists of benthic invertebrates. The species have no economic importance (Karapetkova & Zhivkov, 2006; Golemanski, 2011).

The three studied fish species are included in the IUCN Red List with the category "LC = Least Concern", as well as in the Biological Diversity Act in Bulgaria (Annex II). *C. elongata* and spined loach are included in the Bern Convention (Annex III). The spined loach is included in the Habitats Directive (Annex II). *S. bulgarica* is included in the Red Book of Bulgaria with the category "VU = Vulnerable" (Convention on the conservation of European wildlife and natural habitats, 1982; 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; Golemanski, 2011; IUCN, 2023).

Helminthological studies

The infection was found with two types of helminths - *Asymphylodora tincae* (Modeer, 1790) Lühe, 1909 (class Trematoda) and *Pseudocapillaria tomentosa* (Dujardin, 1845) Moravec, 1987 (class Nematoda) (Table 2). Infection was not found in six specimens of *C. taenia* from Koshava biotope and in two specimens of *C. elongata* from Kudelin biotope. Common helminth species were not found for the two infected loach species.

Table 2. Taxonomic position, synonyms, localization, season, hosts, minimum and maximum value of infection of *Asymphylodora tincae* and *Pseudocapillaria tomentosa*

Helminth species	***Asymphylodora tincae* (Modeer, 1790) Lühe, 1909**	***Pseudocapillaria tomentosa* (Dujardin, 1845) Moravec, 1987**
Taxonomic position	Family Monorchidae Odhner, 1911 Genus *Asymphylodora* Looss, 1899	Family Capillariidae Railliet, 1915 Genus *Pseudocapillaria* Freitas, 1959
Synonyms[1, 2]	*Asymphylodora perlata* (von Nordmann, 1832) Looss, 1899; *Distoma perlatum* von Nordmann, 1832; *Distoma tincae* (Modeer, 1790) Rudolphi, 1809; *Fasciola tincae* Modeer, 1790	*Capillaria amurensis* Finogenova, 1967; *C. tuberculata* (Linstow, 1914) Lewaschoff, 1929; *C. bakeri* Mueller & Van Cleave, 1932; *C. catostomi* Pearse, 1924; *C. gobionina* Lomakin, 1971; *C. leucisci* Hesse, 1923; *C. lewaschoffi* Heinze, 1993; *C. pseudorasborae* Wang, Zhao & Chen, 1978; *C. rutili* Zakhvatkin & Azheganova, 1940; *C. ugui* Yamaguti, 1941; *Skrjabinocapillaria elopichthydis* Wang, 1982; *Trichosoma brevispiculum* Linstow, 1873; *Tr. cyprini* Diesing, 1851; *Tr. tomentosum* Dujardin, 1843
Localization	intestine	intestine (especially in the distal part)
Season	spring	spring
Hosts	*Sabanejewia bulgarica*	*Cobitis elongata*
Minimum and maximum value of infection	2	1

[1]WoRMS (2022). *Asymphylodora tincae* (Modeer, 1790) Lühe, 1909. Accessed at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=744986 on 2022-11-11

[2]Nemys eds. (2022). Nemys: World Database of Nematodes. *Pseudocapillaria (Pseudocapillaria) tomentosa* (Dujardin, 1843) Lomakin & Trofimenko, 1982. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=991382 on 2022-11-11

As. tincae is determinated as a specific parasite of *Tinca tinca* (Linnaeus, 1758), but it has also been reported for the definitive hosts *Abr. brama*; *R. rutilus*, and others. The species is distinguished by a one-year cycle of development and has intermediate hosts - the snails *Bithynia tentaculata* (Linnaeus, 1758) and *Radix auricularia* (Linnaeus, 1758) (Bykhovskaya-Pavlovskaya et al., 1962; Gaevskaya et al., 1975; Kakacheva-Avramova,

1983; Bauer, 1987). Definitive hosts of *Ps. tomentosa* are freshwater fish of the families Cyprinidae, Balitoridae, Cobitidae, Siluridae, Blenniidae, Gobiidae, Percidae, Lotidae, Anguillidae, Esocidae, and others. Intermediate hosts are freshwater oligochaetes (*T. tubifex*, *Limnodrilus hoffmeisteri* Claparède, 1862, *Lumbricus variegatus* (Müller, 1774), and others) and others (Bauer, 1987; Moravec, 2013).

In one of the two examined specimens *C. elongata* from the Danube River (Koshava biotope) one specimen *Ps. tomentosa* was found with mean intensity MI = 1.00; mean abundance MA = 0.50 and prevalence P% = 50.00. From one specimen *S. bulgarica* from the Danube River (Kudelin biotope) two specimens *As. tincae* were isolated. Trematode *As. tincae* had equal mean intensity and mean abundance (MI = MA = 2.00), as well as prevalence P% = 100.00. *As. tincae* stood out by higher ecological indices.

The parasite fauna of the three investigated fish species is poorly studied. *C. elongata* has been reported as a host of *Allocreadium transversale* (Rudolphi, 1802) Odhner, 1901 from the Danube River basin in Bulgaria (Vit River) (Šmiga et al., 2020). *C. taenia* has been reported as a host of *Diplostomum spathaceum* (Rudolphi, 1819) Olsson, 1876, *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908, and others from the Danube River basin in Serbia (Djikanovic et al., 2011). *S. bulgarica* has been reported as the host of *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960 (syn. *Crowcrocaecum skrjabini* (Iwanitzky, 1928) Skrjabin & Koval, 1956) (Margaritov, 1966; Kakacheva-Avramova, 1977; Kakacheva-Avramova et al., 1978), *P. laevis* (Margaritov, 1966; Kakacheva-Avramova et al., 1978), Caryophyllaeidae G. sp., Acanthocephala G. sp. (Margaritov, 1966), and others from the Bulgarian section of the Danube River.

The trematode *As. tincae* identified in the present study was reported in *T. tinca* from the Lake Balaton part of the Danube River basin in Hungary (Molnár & Székely, 1995); in *T. tinca* and *R. rutilus* from rivers on the territory of Serbia (Djikanovic et al., 2011). The species was also discovered in *Abr. brama* from the Bulgarian section of the Danube River (Vetren biotope) (Chunchukova et al., 2016), as well as in *Scardinius erythrophthalmus* (Linnaeus, 1758) from the Srebarna Lake (Margaritov, 1959). The other helminth in this study - the nematode *Ps. tomentosa* was reported in *Barbus barbus* (Linnaeus, 1758) from the Hungarian section of the Danube River (Moravec et al., 1997); in *Ponticola kessleri* (Günther, 1861) (syn. *Neogobius kessleri* Günther, 1861) from the Slovak section of the river (Ondračková et al., 2009; Ondračková et al., 2010); in *Neogobius melanostomus* (Pallas, 1814) from the Austrian section of the Danube River (Ondračková et al., 2010); in *Carassius carassius* (Linnaeus, 1758) from the Danube River basin in Serbia (Djikanovic et al., 2011); in *Carassius gibelio* (Bloch, 1782) and *Pseudorasbora parva* (Temminck & Schlegel, 1846) from reservoirs in Moldova (Gologan, 2020); in *P. kessleri* (Ondračková et al., 2006; Ondračková et al., 2010) and *B. barbus* (Nachev, 2010) from the Bulgarian section of the Danube River.

CONCLUSIONS

As a result of the conducted ecologohel minthological research, *Asymphylodora tincae* (Modeer, 1790) Lühe, 1909 is reported for the first time as a helminth of *S. bulgarica* (Kudelin biotope), and *Pseudocapillaria tomentosa* (Dujardin, 1845) Moravec, 1987 is reported for the first time as a helminth of *C. elongata* (Koshava biotope). The received ecological indices were low, but *As. tincae* stood out with higher ecological indices. *C. elongata* is a new host for *Ps. tomentosa* in Bulgaria. *Ps. tomentosa* has not been reported by *C. elongata* from the Danube River and the river basin from other countries and Bulgaria. *S. bulgarica* is a new host for *As. tincae*. *As. tincae* has not been reported by *S. bulgarica* from the Danube River and its basin, both in other countries and in Bulgaria. Koshava and Kudelin biotopes are new habitats for the found helminth species of *C. elongata* and *S. bulgarica*, respectively.

ACKNOWLEDGEMENTS

We express our gratitude to the Agricultural University - Plovdiv for the opportunity to carry out this research. We thank the Centre of

Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University - Plovdiv for the financial support.

REFERENCES

Bauer, O. (Ed.) (1987). *Key to the Parasites of Freshwater Fishes of the USSR*. Leningrad, RU: Nauka Publishing House (in Russian).

Biological Diversity Act, Promulgated, State Gazette No. 77/9.08.2002

Bykhovskaya-Pavlovskaya, I. E., Gusev, A. V., Dubinina, M. N., Izyumova, T. S., Smirnova, T. S., Sokolovskaya, I. L., Schein, G. A., Shulman, S. S., & Epshchein, V. M. (1962). *Key to the parasite on the freshwater ribeye of the USSR. Moscow - Leningrad*, USSR Academy of Sciences, 200–775 (in Russian).

Chunchukova, M., Shukerova, S., & Kirin, D. (2016). Research of the impact of the river Danube on the Srebarna biosphere reserve by the model ecosystem *Abramin brama* – macroinvertebrates – sediments. *Agricultural Sciences/Agrarni Nauki*, *VIII* (19), 151–158.

Chunchukova, M., & Kirin, D. (2020). New data on the helminth fauna of *Abramis brama* from the Danube river, Bulgaria. *Scientific Papers. Series D. Animal Science*, *LXIII* (2), 477–482.

Chunchukova, M., Kirin, D., & Kuzmanova, D. (2020). Arsenic content in the parasite-host systems: *Pomphorhynchus laevis-Abramis brama* and *Acanthocephalus lucii-Abramis brama*. *Scientific Papers. Series D. Animal Science*, *63*(2), 387–392.

Convention on the conservation of European wildlife and natural habitats, OB L 38, 10.2.1982

Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, OB L 206, 22.7.1992

Djikanovic, V., Paunovic, M., Nikolic, V., Simonovic, P., & Cakic, P. (2011). Parasitofauna of freshwater fishes in the Serbian open waters: a checklist of parasites of freshwater fishes in Serbian open waters. *Reviews in Fish Biology and Fisheries*, *22*(1), 297–324. DOI: 10.1007/s11160-011-9226-6.

Dubinina, M. N. (1948). Parasite fauna of the wild gray goose (*Anser anser*). Parasitol. Sat. Zool. Institute of the Academy of Sciences of the USSR, *12*, 300–351 (in Russian).

Freyhof, J. & Brooks, E. (2011). *European Red List of Freshwater Fishes*. Luxembourg: Publications Office of the European Union.

Gaevskaya, A.V., Gusev, A.V., Deljamure, S.L., Donetc, Z.S., Iskova, N.I., Kornjushin, V.V., Kovaleva, A.A., Margaritov, N.M., Markevitch, A.P., Mordvinova, T.N., Najdenova, N.N., Nikolaeva, V.M., Parukhin, A.M., Pogoreltceva, T.P., Smogorzhevskaja, L.A., Solonchenko, A.I., Shtein, G.A., & Shulman, S.S. (1975). *Key to parasites of vertebrata of the Black and Azov Seas*. Naukovadumka, Kiev, 552 pp. (in Russian).

Golemanski, V. (Ed-in-Chief) (2011). *Red Data Book of the Republic of Bulgaria*. Sofia, BG: Jointedited of the Bulg. Acad of Sci. and Ministry of Environment andWaters, Vol. 2 - Animalia (In Bulgarian).

Gologan, I. (2020). The helminth fauna of some invasive fishes from various natural and artificial water bodies from the Republic of Moldova. *Lucrări Ştiinţifice Seria Medicină Veterinară*, *63*(2), 136–141.

IUCN (2023). The IUCN Red List of Threatened Species. Version 2022-2. <https://www.iucnredlist.org>

Juhásová Ľ., Radačovská A., Bazsalovicsová E., Miklisová D., Bindzárová-Gereľová M., & Králová-Hromadová I. (2019). A study of the endohelminths of the European perch *Perca fluviatilis* L. from the central region of the Danube river basin in Slovakia. *ZooKeys*, *899*, 47–58.

Kakacheva-Avramova, D. (1977). Study on the helminth composition of fish from the Bulgarian part of the Danube River. *Khelmintologiya*, *3*, 20–43 (In Bulgarian).

Kakacheva-Avramova, D., Margaritov, N., & Grupcheva, G. (1978). Fish parasites of Bulgarian part of the Danube River. *Limnology of Bulgarian part of the Danube River*, *Bulg. Acad. Sci*., 250–271 (in Bulgarian).

Kakacheva-Avramova, D. (1983). *Helminths of freshwater fish in Bulgaria*. Izdatelstvo na Balgarskata Akademiya na Naukite, Sofia, 261 pp (in Bulgarian).

Karapetkova, M., & Zhivkov, M. (2006). *Fishes in Bulgaria*. Sofia, BG: GeaLibris, 216 pp (in Bulgarian).

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., Hanzelova, V., Shukerova, S., & Kuzmanova, D. (2014). Biodiversity, Bioindication and Helminth communities of *Abramis brama* (Linnaeus, 1758) from the Danube River and Lake Srebarna, Bulgaria. *Turkish Journal of Agricultural and Natural Sciences*, 727–733.

Kottelat, M., & Freyhof, J. (2007). *Handbook of European freshwater fishes*. Berlin, GE: Publications Kottelat, Cornol and Freyhof Publishing House, 646 pp.

Kuzmanova, D., Chunchukova, M., & Kirin, D. (2019). Helmints and helminth communities of *Squalius cephalus* (Linnaeus, 1758) from Osym river, Bulgaria. *Scientific Papers. Series D. Animal Science*, *62*(1), 456–462.

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

Margaritov, N. (1966). Helminths of the digestive systems and the body cavity of the fish from the Bulgarian section of the Danube River. *Notifications from the Zool. Ins. Museum*, *XX*, 157–173 (In Bulgarian).

Molnár, K., & Székely, C. (1995). Parasitological survey of some important fish species of Lake Balaton. *Parasitologia Hungarica*, *28*, 63–82.

Moravec, F., Konecný, R., Baska, F., Rydlo, M., Scholz, T., Molnár, K., & Schiemer, F. (1997). *Endohelminth fauna of barbel, Barbus barbus (L.), under ecological conditions of the Danube basin in Central Europe*.

Praha, CZ: Academy of Sciences of the Czech Republic.

Moravec, F. (2013). *Parasitic nematodes of freshwater fishes of Europe*. Praha, CZ: Academia Publsihing House.

Nachev, M. (2010). *Bioindication capacity of fish parasites for the assessment of water quality in the Danube River*. PhD these, Sofia

Nemys eds. (2022). Nemys: World Database of Nematodes. *Pseudocapillaria (Pseudocapillaria) tomentosa* (Dujardin, 1843) Lomakin & Trofimenko, 1982. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=991382 on 2022-11-11

Ondračková, M., Trichkova, T., & Jurajda, P. (2006). Present and Historical Occurrence of Metazoan Parasites in *Neogobius kessleri* (Pisces: Gobiidae) in the Bulgarian Section of the Danube River. *Acta Zoologica Bulgarica*, *58*(3), 401–408.

Ondračková, M., Dávidová, M., Blažek, R., Gelnar, M., & Jurajda, P. (2009). The interaction between an introduced fish host and local parasite fauna: *Neogobius kessleri* in the middle Danube River. *Parasitology Research*, *105*, 201–208.

Ondračková, M., Francová, K., Dávidová, M., Polačik, M., & Jurajda, P. (2010). Condition status and parasite infection of *Neogobius kessleri* and *N. melanostomus* (Gobiidae) in their native and non-native area of distribution of the Danube River. *Ecological Research*, 25(4), 857–866.

Šmiga, Ľ., Fedorčák, J., Faltýnková, A., Stefanov, T., Bystrianska, J., Halačka, K., Koščová, L., & Koščo, J. (2020). Endohelminth Parasites in Loaches *Cobitis* spp. (Actinopterygii: Cobitidae), with the First Record of *Allocreadium transversale* (Rudolphi, 1802) (Digenea: Allocreadiidae) in Bulgaria. *Acta Zoologica Bulgarica*, *72*(2), 311–314.

Vostradovsky, J. (1973). *Freshwater fishes*. London, UK: The Hamlyn Publishing Group Limited, 252 p.

WoRMS (2022). *Asymphylodora tincae* (Modeer, 1790) Lühe, 1909. Accessed at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=744986 on 2022-11-11

Zashev, G., & Margaritov, N. (1966). *Diseases of fish*. Sofia, BG: Nauka i izkustvo Publishing House (in Bulgarian).

https://www.google.bg/maps/place/Видин - Google Maps.