A study of the endohelminths of the European perch
*Perca fluviatilis* L. from the central region of the
Danube river basin in Slovakia

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Abstract

The European perch *Perca fluviatilis* L. serves as a host of different endohelminths of Trematoda, Cestoda, Nematoda, and Acanthocephala. Its natural range covers freshwater basins throughout much of Europe, including the Danube. Since information about endohelminths of European perch from this international river basin has been rather sporadic, the parasitological examinations of 700 perch from the central region of the Danube river basin in Slovakia were performed in October 2017 and April 2018. The larval stages of *Triaenophorus nodulosus* (Cestoda) were found in cysts located in the perch liver and adults of *Proteocephalus percae* (Cestoda) were isolated from the intestine. The larval stages of *Eustrongylides* sp. (Nematoda) and metacercariae of *Clinostomum complanatum* (Trematoda), both potential causative agents of fish-borne zoonoses, were found in the musculature. Spatial and seasonal differences in the occurrence of currently detected helminths were discussed with data on biological and environmental conditions of particular sampling site.

Keywords

endoparasites, *Clinostomum complanatum*, *Eustrongylides* sp., Percidae, *Proteocephalus percae*, *Triaenophorus nodulosus*
Introduction

The Danube is the second longest river in Europe shared by 10 European countries, including Germany, Austria, Slovakia, Hungary, Croatia, Serbia, Romania, Bulgaria, Moldova, and Ukraine. The Danube river basin, one of the most international river basins in the world (Liska 2015), is divided into Upper, Middle, and Lower basins (Liška et al. 2008). The largest part is the Middle Basin, which includes the area from Bratislava in Slovakia to the Iron Gate dams at the border of Serbia and Romania (Paunovic et al. 2007).

The Danube represents an important ecosystem with a high biodiversity of aquatic organisms (Tockner et al. 1998). A study on the fish fauna of the entire course of the Danube revealed the presence a high diversity of some 100 fish species, including cyprinids, silurids, esocids, percids, anguillids, and salmonids (Schiemer et al. 2004). Due to its international character and rich fish fauna, the Danube also plays a notable role in the spreading of various parasitic and infectious fish diseases.

Percids represent a so-called promising fish species for a fishery and aquaculture (Kestemont et al. 2015). The European perch, *Perca fluviatilis* Linnaeus, 1758, is an ecologically significant predator and popular sport fish noted for its fighting qualities and taste (Popova and Sytina 1977). It is among the most common and widely distributed members of the Percidae throughout Europe (Giannetto et al. 2012), including the Danube.

The European perch serves as a host for different endohelminths (Trematoda, Cestoda, Nematoda, and Acanthocephala). However, only a few parasitological studies have been conducted on the European perch from the Danube since the 1980s. All of them were in the Lower Basin, in particular in Srebarna Lake (north-eastern Bulgaria), which is connected via an artificial canal to the Bulgarian part of the Danube (Kakacheva-Avramova 1983; Shukerova et al. 2010; Atanasov 2012; Kirin et al. 2013). Since no recent information about endohelminths of the European perch from the Middle Danube is available, parasitological examinations of perch from five selected localities in the Slovak part of the river were performed in two periods of the year. The spatial and seasonal differences in the occurrence of detected endohelminths were discussed with data on biological and environmental conditions of particular sampling site.

Material and methods

Material

The European perch were collected from the central region of the Danube, in particular from four river branches (RB) located next to the main stream and from Šulianske Lake, a gravel pit permanently flooded with water and near the Danube (Fig. 1).

In total, 700 individuals of European perch (length 107–165 mm; 71.4% females and 28.6% males) from all localities were caught by professional fishermen in October 2017 and April 2018. The number of fish obtained during both seasons was approximately equal (October 49.4%; April 50.6%; for more details see Table 2). Incomplete
parasitological necropsy included a detailed examination of the peritoneal cavity, intestine, liver and other abdominal organs. In order to examine the musculature of perch, thin (approximately 5 mm) slices of fillets of whole fish were examined. The skin, gills, and eyes were not investigated. The parasites were washed in a physiological solution and observed under a stereoscopic microscope for morphological identification to genus and/or species level using taxonomic keys (Moravec 1994; Scholz and Hanzelová 1998; Gibson et al. 2002; Kucha et al. 2008).

**Figure 1.** The schematic presentation of sampling sites in Slovak part of the Danube. 1, Karloveské river branch (48°8’46.08"N, 17°3’50.33"E); 2, Starohájske river branch (48°6’11.50"N, 17°7’56.19"E); 3, Jarovecké river branch (48°4’32.34"N, 17°8’23.90"E); 4, Biskupické river branch (48°5’15.45"N, 17°9’44.21"E); 5, Šulianske Lake (47°56’26.66"N, 17°25’42.55"E).
Molecular genotyping

The parasites were rinsed in physiological solution and fixed in 96% ethanol immediately after dissection. Taxonomic identification of the parasites to the species level was performed by molecular genotyping using a partial small subunit of the nuclear ribosomal RNA gene (ssrDNA) as a molecular marker. For PCR amplification and sequencing of ssrDNA, the following universal primers were applied: WormA (5’–GCGAATGGCTCATTAAATCAG–3’) and WormB (5’–CTTGTTACGACTTTTACTTCC–3’) (Littlewood and Olson 2001). Details on PCR amplification, sequencing and sequence analysis were published in Bazsalovicsová et al. (2018). The data obtained were compared with sequences deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/).

Statistical tests

Fisher’s exact test was used to compare the prevalence of endohelminths from the five studied localities between the two seasons. The samples were initially analysed as separate observations of the locality per season for each parasite species, then consequently evaluated independently to the examination timing for each locality. A $p$ value under 0.05 was considered to be significant. Moreover, 95% confidence intervals (CI) were calculated individually for each proportion. The statistical analyses were performed by the Quantitative Parasitology on the Web (Reiczigel et al. 2019).

Results

Of the 700 European perch examined from five sampling sites in the Middle Danube river basin in Slovakia in October 2017 and April 2018, 176 were found to be infected (prevalence 25.1%; CI 22.0–28.5%). Endohelminths were determined in all the studied localities; however, species composition and prevalence varied between different sampling sites and/or examination timing.

Two tapeworms were found in European perch; larval stages of *Triaenophorus nodulosus* (Pallas, 1781) Rudolphi, 1793 (Bothriocephalidea) were found in cysts localized in the liver, and juveniles to adults (at different stages of maturity) of *Proteocephalus percae* (Müller, 1780) (Proteocephalidea) were isolated from the pyloric caeca. In the musculature, the larval stages of the nematodes of the genus *Eustrongylides* Jägerskiöld, 1909 (Dioctophymatoidea) and metacercariae of the fluke *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 (Diplostomida) were detected.

To confirm the taxonomic status of all detected species, ssrDNA was applied as the molecular marker for genotyping. After PCR amplification, a 730 bp fragment was obtained, sequenced and compared with sequences of respective species deposited in the GenBank. The ssrDNA sequence of *T. nodulosus* from our study was 100% identical with *T. nodulosus* from pike (*Esox lucius*) from Scotland (GenBank Accession number KR780923; Bra-
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bec et al. 2015), and the one of *P. percae* was 100% identical with *P. percae* from European perch from Switzerland (KX768934; Scholz et al. 2017). The nematode *C. complanatum* corresponded (100% identity) with *C. complanatum* from Italy (FJ609420; Gustinelli et al. 2010). The sequence of *Eustrongylides* sp. was 99.1% identical with *Eustrongylides* sp. from dwarf snakehead (*Channa gachua*) from India (MG696298; unpublished).

**Note:** Although more than 20 species of the genus *Eustrongylides* were originally described, the validity of many of them is disputable (Eberhard and Ruiz-Tiben 2014). A revision of the genus revealed that there are three valid species: the type species *Eustrongylides tubifex* (Nitzsch & Rudolphi, 1819) Jägersköld, 1909; *Eustrongylides ignotus* Jägersköld, 1909; and *Eustrongylides excisus* Jägersköld, 1909 (Measures 1988). Although *E. excisus* and *E. tubifex* have previously been reported from perch in the Lower Danube (Table 1), there are no data on any DNA region of both nematodes available in the GenBank database. The sequences obtained in the current study corresponded to the sequence data on species assigned as *Eustrongylides* sp.

*Triaenophorus nodulosus* (larvae)

The mean intensity of infection (MI) for *T. nodulosus* from all five localities ranged between 1.0 and 9.5 (Table 2). The overall highest prevalence was observed for *T. nodulosus* in perch from the Biskupické RB, with higher values in October (P = 49%) than in April (P = 23%). On the contrary, larvae of this tapeworm were not detected in Šulianske Lake irrespective of the season (Fig. 2; Table 2). There were no statistically significant differences between sampling periods in all studied localities, except for the Biskupické RB (p < 0.05) (Table 2).

*Proteocephalus percae* (adult)

The MI for this intestinal tapeworm was 2.3 and 4.0 (Table 2). It was detected in two out of five localities, in particular in the Karloveské RB (P = 7.0%) and Starohájske RB (P = 11.1%); at both localities *P. percae* was present only in spring (Fig. 2, Table 2).

*Clinostomum complanatum* (metacercariae)

The mean intensity of infection ranged between 1.0–3.4 (Table 2). The highest prevalence (45%) was detected in the Biskupické RB in October and markedly lower values (P = 10%) were recorded in April (Fig. 2; Table 2). A similar seasonal pattern was observed in Šulianske Lake (October, P = 14%; April, P = 6%). While there was high statistical support for the results in the Biskupické RB (p < 0.001), data detected in Šulianske Lake were statistically nonsignificant (Table 2). Opposite results were observed in the three remaining localities, where metacercariae of *C. complanatum* were not detected in October but were present in April (Fig. 2; Table 2).
**Table 1.** Summary of the literature data (1980–2019) of endohelminths detected in European perch *Perca fluviatilis* L. in the Danube.

| Parasite                  | Locality                     | Season  | No.  | P (%) | Dev. stage | References                  |
|---------------------------|------------------------------|---------|------|-------|------------|------------------------------|
| **CESTODA**               |                              |         |      |       |            |                              |
| *Proteocephalus percae*   | Srebarna Lake, NE Bulgaria  | autumn  | 60   | 3.3   | A          | Shukerova et al. 2010        |
|                           |                              | spring  | 60   | 1.7   | A          | Shukerova et al. 2010        |
| **TREMATODA**             |                              |         |      |       |            |                              |
| *Bolboforus confusus*     | Srebarna Lake, NE Bulgaria  | spring  | 60   | 3.3   | M          | Shukerova et al. 2010        |
|                           |                              | summer  | 60   | 10.0  | M          | Shukerova et al. 2010        |
|                           |                              | autumn  | 60   | 1.7   | M          | Shukerova et al. 2010        |
| **Diplostomum**           | Srebarna Lake, NE Bulgaria  | autumn  | 60   | 1.1   | M          | Shukerova et al. 2010        |
| *pseudopathaceum*         | River Danube, Bulgaria       | n.a.    | 40   | 20.0  | M          | Atanasov 2012                |
| **Diplostomum spathaceum**| Srebarna Lake, NE Bulgaria  | spring  | 60   | 3.3   | M          | Shukerova et al. 2010        |
|                           |                              | summer  | 60   | 1.7   | M          | Shukerova et al. 2010        |
|                           |                              | autumn  | 60   | 1.7   | M          | Shukerova et al. 2010        |
| *Ichthyocotylurus pileatus*| Srebarna Lake, NE Bulgaria | summer  | 60   | 3.3   | M          | Shukerova et al. 2010        |
|                           | Srebarna Lake, NE Bulgaria  |         |      |       |            |                              |
| *Posthodiplostomum cuticola*| Srebarna Lake, NE Bulgaria | summer  | 60   | 1.7   | M          | Shukerova et al. 2010        |
| **Tylodelphys clavata**   | Srebarna Lake, NE Bulgaria  | spring  | 60   | 56.7  | M          | Shukerova et al. 2010        |
|                           |                              | summer  | 60   | 81.7  | M          | Shukerova et al. 2010        |
|                           |                              | autumn  | 60   | 86.7  | M          | Shukerova et al. 2010        |
| **NEMATODA**              |                              |         |      |       |            |                              |
| *Contracaecum microcephalum*| Srebarna Lake, NE Bulgaria | autumn  | 60   | 3.3   | L          | Shukerova et al. 2010        |
| *Eustrongylides excius*   | Srebarna Lake, NE Bulgaria  | spring  | 60   | 8.3   | L          | Shukerova et al. 2010        |
|                           |                              | summer  | 60   | 10.0  | L          | Shukerova et al. 2010        |
|                           |                              | autumn  | 60   | 23.3  | L          | Shukerova et al. 2010        |
|                           | River Danube, Bulgaria       | n.a.    | 40   | 7.5   | L          | Atanasov 2012                |
| *Eustrongylides tubifex*  | Srebarna Lake, NE Bulgaria  | autumn  | n.a. | 1.0   | L          | Kirin et al. 2013            |
|                           | River Danube, Bulgaria       | n.a.    | 100  | 100   | L          | Kirin et al. 2013            |
| *Rhaphidascaris acus*     | Srebarna Lake, NE Bulgaria  | autumn  | 60   | 1.7   | L          | Shukerova et al. 2010        |
|                          |                              | n.a.    | n.a. | n.a.  | n.a.       | Kakacheva-Avramova 1983      |
| **ACANTHOCEPHALA**        |                              |         |      |       |            |                              |
| *Acanthocephalus lucii*   | Srebarna Lake, NE Bulgaria  | spring  | 60   | 1.7   | A          | Shukerova et al. 2010        |
| *Acanthocephalus anguillic*| Srebarna Lake, NE Bulgaria  | spring  | 60   | 1.7   | A          | Shukerova et al. 2010        |

No. number of fish examined, P prevalence, Dev. stage developmental stage, NE north-eastern, n.a. not available, A adults, L larvae, M metacercariae.

**Eustrongylides sp. (larvae)**

The MI values ranged between 1.0–2.8 (Table 2). The larvae of *Eustrongylides* sp. were detected in perch musculature at the highest prevalence in the Jarovecké RB, where no striking differences were detected between October (P = 26%) and April (P = 24%) (Fig. 2; Table 2). A similar prevalence (P = 22%) was detected in Šulianske Lake in spring, while lower prevalence (P = 5%) was recorded in October (Fig. 2; Table 2). Larvae of *Eustrongylides* sp. were not detected in the Karloveské RB (Fig. 2; Table 2).
Table 2. Statistical data on detected endohelminths of European perch *Perca fluviatilis* L. from studied localities in the Danube river basin, Slovakia.

| Locality         | TE    | No. | *Triaenophorus nodulosus* (Cestoda) | *Proteocephalus percae* (Cestoda) |
|------------------|-------|-----|----------------------------------|----------------------------------|
|                  |       |     | IF | MI (max) | P (%) | FET | 95% CI | IF | MI (max) | P (%) | FET | 95% CI |
| Karloveské RB    | Oct/17| 29  | 2  | 9.5 (15) | 7      | ns  | 1–23   | 0  | –        | –    | –    | –     |
|                  | Apr/18| 57  | 3  | 1.0 (1)  | 5      | ns  | 1–15   | 4  | 4.0 (4)  | 7    | ns  | 2–17  |
| Starohájske RB   | Oct/17| 143 | 3  | 3.0 (4)  | 2.1    | ns  | 0.4–6.0| 0  | –        | –    | –    | –     |
|                  | Apr/18| 171 | 10 | 2.8 (12) | 5.8    | ns  | 2.8–10.5| 19 | 2.3 (8)  | 11.1  | ***  | 6.8–16.8 |
| Jarovecké RB     | Oct/17| 70  | 3  | 1.3 (2)  | 4      | ns  | 1–12   | 0  | –        | –    | –    | –     |
|                  | Apr/18| 49  | 2  | 5.0 (9)  | 4      | ns  | 5–14   | 0  | –        | –    | –    | –     |
| Biskupické RB    | Oct/17| 67  | 33 | 7.1 (20) | 49     | *   | 37–62  | 0  | –        | –    | –    | –     |
|                  | Apr/18| 31  | 7  | 6.4 (12) | 23     | *   | 10–41  | 0  | –        | –    | –    | –     |
| Šulianske Lake   | Oct/17| 37  | 0  | –       | –      | –   | –      | 0  | –        | –    | –    | –     |
|                  | Apr/18| 46  | 0  | –       | –      | –   | –      | 0  | –        | –    | –    | –     |
| In total         |       | 700 | 63 | 5.6 (20) | 9.0    | –   | 7.0–11.4| 23 | 2.3 (8)  | 3.3   | –    | 2.1–4.9 |

| Locality         | TE    | No. | *Clinostomum complanatum* (Trematoda) | *Eustrongylides* sp. (Nematoda) |
|------------------|-------|-----|-------------------------------------|-------------------------------------|
|                  |       |     | IF | MI (max) | P (%) | FET | 95% CI | IF | MI (max) | P (%) | FET | 95% CI |
| Karloveské RB    | Oct/17| 29  | 0  | –       | –      | –   | –      | 0  | –        | –    | –    | –     |
|                  | Apr/18| 57  | 2  | 2.0 (2) | 4      | ns  | 1–12   | 0  | –        | –    | –    | –     |
| Starohájske RB   | Oct/17| 143 | 0  | –       | –      | –   | –      | 6  | 1.3 (2)  | 4.2   | ns  | 1.6–8.9 |
|                  | Apr/18| 171 | 5  | 1.0 (1) | 2.9    | ns  | 1.0–6.7| 11 | 1.9 (10) | 6.4   | ns  | 3.3–11.2 |
| Jarovecké RB     | Oct/17| 70  | 0  | –       | –      | –   | –      | 18 | 1.4 (5)  | 26    | ns  | 16–38  |
|                  | Apr/18| 49  | 6  | 2.0 (6) | 12     | **  | 5–25   | 12 | 2.2 (7)  | 24    | ns  | 13–39  |
| Biskupické RB    | Oct/17| 67  | 30 | 3.4 (23)| 45     | *** | 33–57  | 5  | 1.0 (1)  | 8     | ns  | 2–17   |
|                  | Apr/18| 31  | 3  | 2.0 (2) | 10     | *** | 2–26   | 1  | 1.0 (1)  | 3     | ns  | 0–17   |
| Šulianske Lake   | Oct/17| 37  | 5  | 1.6 (4) | 14     | ns  | 4–28   | 2  | 1.0 (1)  | 5     | ns  | 7–18   |
|                  | Apr/18| 46  | 3  | 1.0 (1) | 6      | ns  | 1–18   | 10 | 2.8 (7)  | 22    | ns  | 11–36  |
| In total         |       | 700 | 54 | 2.6 (23) | 7.7    | –   | 5.8–9.9| 65 | 1.8 (10) | 9.3   | –    | 7.2–11.7 |

RB river branch, TE timing of examination, Oct/17 October 2017, Apr/18 April 2018, No. number of fish examined, IF number of infected fish, MI mean intensity of infection, max maximum number of parasites, P prevalence, FET Fisher’s exact test of seasonal differences in prevalence for each locality separately, 95% CI confidence interval, ns nonsignificant, *p < 0.05, **p < 0.01, ***p < 0.001
Discussion

Over the last four decades, several species of flukes and nematodes, two species of thorny-headed worms and single tapeworm have been found in European perch from the Danube (for details, see Table 1 and references therein). In the current study, only four endohelmints were detected in perch from the central region of the river. Metacercariae of *C. complanatum* were detected for the first time, while *P. percae* and *Eustrongylides* sp. were previously found in Srebarna Lake in north-eastern Bulgaria (Shukerova et al. 2010; Kirin et al. 2013). The only record of the presence of *T. nodulosus* in perch from the Danube was published more than 60 years ago (Dyk 1955). Spatial and seasonal differences in the occurrence of currently detected helminths could be explained by diverse environmental conditions of particular sampling site and by an availability of suitable definitive hosts.

The occurrence of *T. nodulosus* in the studied localities was rather diverse; it was absent in Šulianske Lake, while low values of prevalence were documented in Karloveské, Starohájske and Jarovecké RB. The highest prevalence was detected in the Biskupické RB, a branch of the river about 20 m wide and connected to the Danube by an artificial channel (Jursa 2003). Water in the stream branch has rich fish diversity, and it is regularly restocked with various fish species, including perch (second intermediate host) and pike (definitive host of *T. nodulosus*). The high prevalence of *T. nodulosus* in this particular RB may be related to the fact that 5000 individuals of pike were restocked in the Biskupické RB in December 2015 (http://cokdezakolko.sk/category/zarybnenie/; in Slovak).

The prevalence of *T. nodulosus* in the Biskupické RB was significantly higher in autumn. On the contrary, no evident seasonal variation was detected in three other stud-
ied localities. Since plerocercoids can live in the intermediate fish host up to three years, little or no seasonal variations have been previously detected in periodicity of *T. nodulosus* in perch. Besides, the dynamics of infections and maintenance of plerocercoids in fish may vary considerably from water to water (Chubb 1980 and references therein).

The second tapeworm detected in the current work, *P. percae*, was present in Karloveské and Starohájske RB only in spring (April). Similar seasonal dynamics with the maximum values of prevalence in March and April were also observed by Scholz (1986) and Chubb (1982), respectively.

The two remaining helminths, *C. complanatum* and *Eustrongylides* sp., utilize birds as definitive hosts. The Protected Bird Area of the Danube floodplain is a refuge for tens of thousands of birds; it is an internationally important breeding area, nesting site, migration corridor, and wintering place of migratory and resident birds, such as mallard, great crested grebe, cormorant, black stork and other long-necked wading birds, which serve as definitive host of the above species. This has evidently played an important role in a broad spatial distribution of both endohelminths; while *C. complanatum* was detected in all five studied localities, *Eustrongylides* sp. was absent only in Karloveské RB.

Whereas birds are preferable hosts of *C. complanatum*, humans can be incidentally infected by eating raw or undercooked freshwater fish infected by *C. complanatum* metacercariae (Soylu 2013), causing parasitic pharyngitis and laryngitis (Gaglio et al. 2016). Human infections are rather rare and have occurred mainly in Asian countries (e.g. Korea) with a tradition of eating raw fish (Kim et al. 2019).

*Eustrongylides* sp. may also pose a public health risk to consumers of raw or undercooked fish, such as perch (Branciari et al. 2016). Human infections have been recorded mainly in Asia (Ljubojevic et al. 2015) or Africa (Eberhard and Ruiz-Tiben 2014). Although humans are not frequent hosts for species of *Eustrongylides*, it is known that this fish-borne zoonosis can cause gastritis and intestinal perforation in occasionally infected human. According to the recommendations of the European Commission, food producers should visually examine fish products before their release to the market (Branciari et al. 2016). Since larvae of species of *Eustrongylides* are typically large and are conspicuously red, they are easily differentiated from the fish tissue, even by visual inspection.

A potential risk of transmission of *C. complanatum* and *Eustrongylides* sp. from perch to humans in Europe is very limited, although it can not be absolutely excluded. A good example is diphyllobothriosis, fish-borne zoonosis, which re-emergence in the subalpine region was due to increased popularity of raw perch dishes (Wicht et al. 2009).

The Danube and its adjacent floodplain forests are characterized by rich aquatic and terrestrial faunas. However, anthropogenic activities, such as hydropower dams (Schiebler et al. 2004) may influence diversity and number of aquatic species (Guti 1992). The data on fish parasites from the Danube are, in general, scarce. Since some information are rather old and require updates, up-to-date surveys are necessary for accurate knowledge on fish parasites from this dynamically changing aquatic environment.
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References

Atanasov G (2012) Fauna, morphology and biology on the endohelminths of fish from Bulgarian part of the Danube river. PhD thesis, Sofia, Bulgaria, Bulgarian Academy of Science. [In Bulgarian]
Bazsalovicsová E, Králová-Hromadová I, Xi BW, Štefka J (2018) Tour around the globe: the case of invasive tapeworm Atractolytocestus huronensis (Cestoda: Caryophyllidea), a parasite of common carp. Parasitology International 67: 366–374. https://doi.org/10.1016/j.parint.2018.02.004
Brabec J, Waeschenbach A, Scholz T, Littlewood DTJ, Kuchta R (2015) Molecular phylogeny of the Bothriocephalidea (Cestoda): molecular data challenge morphological classification. International Journal for Parasitology 45: 761–771. https://doi.org/10.1016/j.ijpara.2015.05.006
Branciari R, Ranucci D, Miraglia D, Valiani A, Veronesi F, Urbani E, Lo Vaglio G, Pascucci L, Franceschini R (2016) Occurrence of parasites of the genus Eustrongylides spp. (Nematoda: Dioctophymatidae) in fish caught in Trasimeno Lake, Italy. Italian Journal of Food Safety 5: 206–209. https://doi.org/10.4081/ijfs.2016.6130
Chubb JC (1980) Seasonal occurrence of helminths in freshwater fishes. Part III. Larval Cestoda and Nematoda. In: Lumsden WHR, Müller R, Baker JR (Eds) Advances in Parasitology 18. Academic Press, London, 1–120. https://doi.org/10.1016/S0065-308X(08)60398-X
Chubb JC (1982) Seasonal occurrence of helminths in freshwater fishes. Part IV. Adult Cestoda, Nematoda and Acanthocephala In: Lumsden WHR, Müller R, Baker JR (Eds) Advances in Parasitology 20. Academic Press, London, 1–292. https://doi.org/10.1016/S0065-308X(08)60539-4
Dyk V (1955) Present stage of investigation of the parasites of Slovak fishes. Biologia 10: 162–172.
Eberhard ML, Ruiz-Tiben E (2014) Case report: cutaneous emergence of Eustrongylides in two persons from South Sudan. American Journal of Tropical Medicine and Hygiene 90: 315–317. https://doi.org/10.4269/ajtmh.13-0638
Gaglio G, Reina V, Caffara M, Gjurcevic E, Iaria C, Marino F (2016) Risk of introduction of Clinostomum complanatum (Digenea: Clinostomidae) to Sicily through use of Cobitis bileatea (Canestrini, 1865) as live baits. Bulletin of the European Association of Fish Pathologists 36: 105–110.
Giannetto D, Carosi A, Franchi E, Pecchillo G, Pompei L, Lorenzoni M (2012) Assessing the impact of non-native freshwater fishes on native species using relative weight. Knowledge and Management of Aquatic Ecosystems 404: 03. https://doi.org/10.1051/kmae/2011081
Endohelminths of European perch from Danube River

Gibson DI, Jones A, Bray RA (2002) Keys to the Trematoda. Vol 1. CABI Publishing and The Natural History Museum, London, 521 pp. https://doi.org/10.1079/9780851995472.0000

Gustinelli A, Caffara M, Florio D, Otachi EO, Wathuta EM, Fioravanti ML (2010) First description of the adult stage of Clinostomum cutaneum Paperna, 1964 (Digenea: Clinostomidae) from grey herons Ardea cinerea L. and a redescription of the metacercaria from the Nile tilapia Oreochromis niloticus niloticus (L.) in Kenya. Systematic Parasitology 76: 39–51. https://doi.org/10.1007/s11230-010-9231-5

Guti G (1992) The population density of perch, Perea fluviatilis L. in the Cikola backwater system of the river Danube, Hungary. Hydrobiologia 242: 195–198. https://doi.org/10.1007/BF00019968

Jursa M (2003) Interesting findings of macrophytes from 3 aquatic biotopes of Bratislava territory. Bulletin Slovenskej botanickej spoločnosti 25: 115–120. [In Slovak]

Kakacheva-Avramova D (1983) Helminths of freshwater fishes in Bulgaria. Bulgarian Academy of Sciences, Sofia, 261 pp. [In Bulgarian]

Kestemont P, Dabrowski K, Summerfelt RC (2015) Biology and culture of percid fishes. Springer, Dordrecht, 901 pp. https://doi.org/10.1007/978-94-017-7227-3

Kim H, Cho S, Oh H, Byeon HK (2019) A case of unexpected Clinostomum complanatum infection initially presenting as foreign body in pharynx. Korean Journal of Parasitology 57: 175–177. https://doi.org/10.3347/kjp.2019.57.2.175

Kirin D, Hanzelová 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 56: 333–340.

Kuchta R, Scholz T, Brabec J, Bray RA (2008) Suppression of the tapeworm order Pseudophyllidea (Platyhelminthes: Eucestoda) and the proposal of two new orders, Bothriocephalidea and Diphyllobothriidea. International Journal for Parasitology 38: 49–55. https://doi.org/10.1016/j.ijpara.2007.08.005

Liska I (2015) The Danube River Basin. The Handbook of Environmental Chemistry, vol. 39. Springer, Berlin, 523 pp. https://doi.org/10.1007/978-3-662-47739-7

Ljubojevic D, Novakov N, Djordjevic V, Radosavljevic V, Pelic M, Cirkovic M (2015) Potential parasitic hazards for humans in fish meat. Procedia Food Science 5: 172–175. https://doi.org/10.1016/j.profoo.2015.09.049

Measures LN (1988) Revision of the genus Eustrongylides Jägersköld, 1909 (Nematoda: Dioctophymatoidea) of piscivorous birds. Canadian Journal of Zoology 66: 885–895. https://doi.org/10.1139/z88-131

Moravec F (1994) Parasitic Nematodes of Freshwater Fishes of Europe. Academia, Praha, 470 pp.

Paunovic MM, Jakovev-Todorovic DG, Simic VM, Stojanovic B, Cakic PD (2007) Macrinivertebrates along the Serbian section of the Danube River (stream km 1429–925). Biologia 62: 214–221. https://doi.org/10.2478/s11756-007-0032-5
Popova OA, Sytina LA (1977) Food and feeding relations of Eurasian perch (*Perca fluviatilis*) and pikeperch (*Stizostedion lucioperca*) in various waters of the USSR. Journal of the Fisheries Research Board of Canada 34: 1559–1570. https://doi.org/10.1139/f77-219

Reiczigel J, Marozzi M, Fábián I, Rózsa L (2019) Biostatistics for parasitologists – a primer to quantitative parasitology. Trends in Parasitology 35: 277–281. https://doi.org/10.1016/j.pt.2019.01.003

Schiemer F, Guti G, Keckeis H, Staras M (2004) Ecological status and problems of the Danube and its fish fauna: a review. In: Welcomme RL, Petir R (Eds) Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries, Phnom Penh, Cambodia, 11–14 February 2003. Bangkok, Food and Agriculture Organization of the United Nations & The Mekong River Commission, Thailand, 273–299.

Scholz T (1986) Observations on the ecology of five species of intestinal helminths in perch (*Perca fluviatilis*) from Mácha lake fishpond system, Czechoslovakia. Věstník československé společnosti zoolohické 50: 300–320.

Scholz T, Hanelová V (1998) Tapeworms of the Genus *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae), Parasites of Fishes in Europe. Academia, Praha, 118 pp. https://doi.org/10.2307/3285724

Scholz T, de Chambrier A, Shimazu T, Ermolenko A, Wæschenbach A (2017) Proteocephalid tapeworms (Cestoda: Onchoprotocephalidea) of loaches (Cobitoidea): Evidence for monophyly and high endemicity of parasites in the Far East. Parasitology International 66: 871–883. https://doi.org/10.1016/j.parint.2016.09.016

Shukerova S, Kirin D, Hanelová V (2010) Endohelminth communities of the perch, *Perca fluviatilis* (Perciformes, Percidae) from Srebarna Biosphere Reserve, Bulgaria. Helminthologia 47: 99–104. https://doi.org/10.2478/s11687-010-0016-9

Soylu E (2013) Metazoan parasites of perch *Perca fluviatilis* L. from Lake Sığırcı, Ipsala, Turkey. Pakistan Journal of Zoology 45: 47–52.

Tockner K, Schiemer F, Ward JV (1998) Conservation by restoration: the management concept for a river-floodplain system on the Danube river in Austria. Aquatic Conservation 8: 71–86. https://doi.org/10.1002/(SICI)1099-0755(199801/02)8:1%3C71::AID-AQC265%3E3.0.CO;2-D

Wicht B, Limoni C, Peduzzi R, Petrinò O (2009) *Diphyllobothrium latum* (Cestoda: Diphyllobothriidea) in perch (*Perca fluviatilis*) in three sub-alpine lakes: influence of biotic and abiotic factors on prevalence. Journal of Limnology 68: 167–173. https://doi.org/10.4081/jlimnol.2009.167