

Research Note

Title: The first determination of *Eustrongylides excisus* Jägerskiöld, 1909 - larvae (Nematoda: Dioctophymatidae) in the pike-perch *Sander lucioperca* in Vojvodina (Serbia)

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Summary

Twenty-one specimens of pike-perch (*Sander lucioperca*) were caught in the Danube-Tisa-Danube Canal in the city area of Novi Sad for parasitological examination. The presence of nematodes in the muscles was revealed in three fish. The parasites were identified to belong to the species *Eustrongylides excisus*, for which the pike-perch is a paratenic host. This finding represents the first determination of the larvae in the pike-perch in Serbia. The pike-perch is infected by ingestion of benthos- or plankton-eating fishes, the second intermediate hosts harbouring the fourth-stage nematode larvae. *E. excisus* is pathogenic to humans, who may be infected by consuming raw or undercooked fish.

Keywords: *E. excisus*; pike-perch; eustrongylidosis

Introduction

Nematodes of the genus *Eustrongylides*, including *E. excisus*, are the causative agents of eustrongylidosis, which may lead to the death of nestlings in nests in the vicinity of river banks, especially herons, but also other wading bird species: egrets, spoonbills, cormorants, coots, eagles, ducks, geese, gulls, even passerines (Moravec, 1994; Spalding & Forrester, 2008; Cole, 2009). In the infected birds of all ages pale mucous membranes, ataxia, lethargy, depression and emaciation have been described (Spalding & Forrester, 2008). Lesions in the proventriculus wall produced by *E. excisus* can be heavy, due to the pathogenic effects of the nematodes coiled in a capsule which project from the serosal surface. During the course of the infection lesions are replaced by connective tissue. Having in mind that the nematode eggs remain viable and infective up to two years and the larvae can survive in the intermediate host for more than a one-year period, it is well documented that the parasites have developed a successful development strategy (Cole, 2009). On arrival into the definite host the development is promptly completed. The

life cycle includes four stages from the egg to sexual maturity. The first-stage larva develops in the egg passed by the infected bird faeces and is ingested by aquatic oligochaetes *Lumbriculus variegatus*, *Tubifex tubifex* or *Limnodrilus* sp., which are the first intermediate hosts. In the coelom or the organs of the oligochaete the second and third larval stages develop. The second intermediate host is some of the plankton- and benthos-eating fish species, e.g. *Fundulus* or *Gambusia*, *Neogobius*, as well as certain species of the Cyprinidae family, e.g. *Carassius carassius* (Pazooki *et al.*, 2007) and *Rutilus kutum* (Sattari & Mokhayer, 2005; Mohammad *et al.*, 2011) which on ingestion of infected oligochaetes serve as second intermediate hosts. The infection of fishes, although being intermediate hosts, may be even more deleterious, resulting in massive diseases (Moravec, 1994). The third-stage larvae transform and moult into the fourth and remain in the fish, most frequently in the muscles, to be ingested by wading birds, the definite hosts (Moravec, 1994; Spalding & Forrester, 2008; Cole, 2009). Predators, such as the pike-perch, on ingestion of infected fishes become paratenic hosts to the nematodes and are capable of infecting birds. This role can also be taken by amphibian and reptile species. In Turkey *Eustrongylides excisus* larvae were detected even in the frog (*Rana ridibunda*) (Saglam & Arikan, 2006) and in the dice snake *Natrix tessellata* (Carlsson *et al.*, 2011). On ingestion by definite hosts the nematode larvae promptly reach sexual maturity and in only 10 – 17 days begin laying eggs (Moravec, 1994; Spalding & Forrester, 2008; Cole, 2009).

The pike-perch is a dweller of rivers and lakes of the basins of the Baltic, Black, Caspian, Aral, and the Sea of Azov. It is also found in the lakes in Finland as far to the north as 64° N. In Serbia, pike-perch is found in the Black sea basin and in all water bodies across Vojvodina Province (Vuković & Ivanović, 1971). According to current literature, as second intermediate hosts gobiid fishes from the

north-western Black sea (Kvach, 2005), Bulgaria and Austria (Francová *et al.*, 2011) were registered, as well as *Aphanius mento* from Turkey (Aydoğdu *et al.*, 2011), *Carassius carassius* (Pazooki *et al.*, 2007) and *Rutilus kutum* (Mohammad *et al.*, 2011) from Iran. Furthermore, *Eustrongylides* larvae were registered in Romania, Hungary, Bulgaria and Slovak Republic, systems of the rivers Danube, Dnieper, Dniester and Volga in the former USSR, central Asia and China (Moravec, 1994). Given the afore mentioned, the aim of the present work was to gain insight into the presence of parasite species in pike-perch in Serbia.

Material and methods

Twenty-one specimens of the pike-perch (*Sander lucioperca*) were caught on the Danube-Tisa-Danube Canal in the city area of Novi Sad (45° 15' 6" N and 19° 50' 12" E) in spring 2012. The fish body mass ranged from 250 g to 500 g.

On comprehensive parasitological examination nematodes were revealed in the muscles of three fish. The nematodes were isolated and conserved in 70 % ethanol. Prior to identification they were processed in lactic acid. Specimens were examined under Leica MZ-16 stereomicroscope and Zeiss Axio Imager A1 light microscope. The determination of species was completed on morphometric analysis and according to keys provided by Bauer (1987), Moravec (1994) and Anderson (2000).

Results

Upon completion of parasitological investigation of twenty-one pike-perch, nematode larvae were diagnosed in the abdominal muscles in three specimens, contributing to the prevalence of infection of 14 %. Eight nematodes were extracted and isolated. It has been verified that the nematode larvae belong to *Eustrongylides excisus* species.

The description of the nematode larvae

Order	Enoplida
Sub-order	Diectophymina
Super-family	Diectophymatoidea
Family	Diectophymatoidea
Genus	<i>Eustrongylides</i>

Species *Eustrongylides excisus* Jägerskiöld, 1909

The definitive hosts of the nematode species are cormorants (*Phalacrocorax carbo*, *P. pygmaeus*) in which it parasitizes in the proventriculus wall (Moravec, 1994).

The larvae detected were reddish in colour. Their body length ranged from 27 mm to 40 mm, which corresponds to the size given by Bauer (1987), Moravec (1994) and Anderson (2000), whilst the values of body width were somewhat higher, from 0.2 to 0.35 mm. Characteristically, there were 12 papillae at the anterior end of the body, set in two circles, each containing six papillae. The papillae in the inner circle were a little more elongated (Figure 1A and 1B). The oral cavity was 0.099 mm long (Fig. 1C), and the length of the oesophagus ranged from 2.9 mm to 5 mm. The anal aperture was located terminally (Fig. 1D) which corresponds to the literature data (Bauer, 1987; Moravec, 1994).

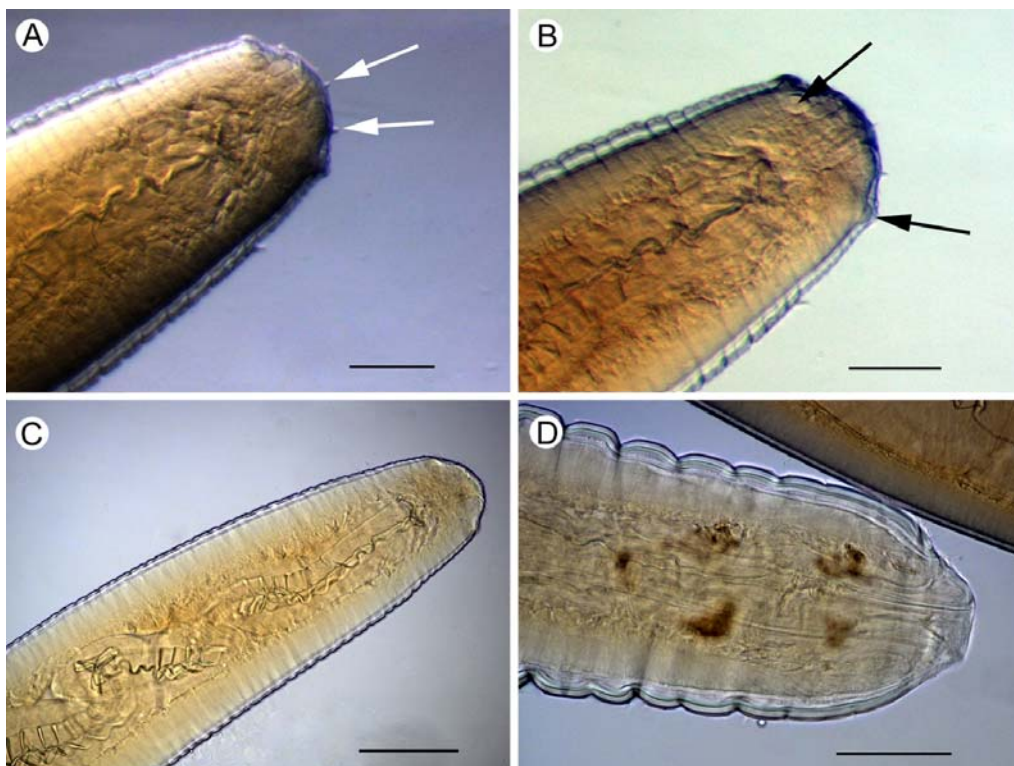


Fig. 1. *Eustrongylides excisus* larvae. A) Inner circle of the finger shaped papillae at the anterior body end, B) Exterior circle of the nipple shaped papillae at the anterior body end, C) Anterior part of the body, D) Posterior part of the body; A – B) Stereomicroscope, C – D) Light microscope; Scale bars: A – B) 1 mm, C – D) 0.2 mm

Discussion

The occurrence of *Eustrongylides excisus* is important from several aspects. First, as was mentioned before, this is a species which leads to the possibly deleterious infection in wading birds. High mortality has been noted in nestlings, significantly higher than in adults. Although the symptoms are not characteristic, certain problems occurring in infected birds can imply that the infection exists. In the early stages of the infection the birds shake their heads, suffer deglutition problems and occasionally vomit. In the proventriculus, ventriculus and the intestines nematodes incorporated in the wall are easily notable (Cole, 2009).

In fishes, which are intermediate hosts, the larvae develop in muscles and are found either free in the body cavity, or encapsulated in the liver or other visceral organs. Frequently, large numbers of comparatively long nematode larvae may be present, thus leading to the enlargement of the abdomen. Infected fishes usually are the prey for piscivorous fish species such as pike-perch. On the ingestion of the prey, the predator itself becomes infected. From its digestive tube the larvae penetrate into its muscles and other organs, which they may seriously damage (Yanong, 2009). According to Moravec (1994), the larvae are especially pathogenic to predator fish species, where they are capable of doing severe damage to various organs. In young fish large scars are readily visible at the prior location of the parasites (muscles, gastric and intestinal wall), sometimes even a complete destruction of the kidneys, or inflammatory lesions. The disease in fish caused by *Eustrongylides* larvae may rather frequently become widespread (Moravec, 1994).

Although the species of *Eustrongylides* genus are typically not hosted by humans, they may become infected on consuming undercooked fish (Mohammad *et al.*, 2011). The nematodes cannot reach maturity in man, but remain in the fourth stage of larval development. The symptoms indicating the infection include gastritis and the perforation of intestines. The only possible means of cure is surgical removal of larvae (Cole, 2009).

The larvae of *Eustrongylides* sp. were diagnosed in the round goby, *Neogobius melanostomus*, at various locations in the Danube (Francova *et al.*, 2011). In addition, in the lake Srebarna, which is connected to the Danube River by a canal, Shukerova *et al.* (2010) found, besides other helminths, the larval stages of *E. excisus* in the European perch, *Perca fluviatilis*. These data have proven the presence of the larvae in fishes in the flow of the Danube. Thus, it is also expected for them to be found in the Danube-Tisa-Danube canal immediately before it meets the Danube River. Satari and Mokhayer (2005) confirmed the occurrence of *E. excisus* in a predator species, that is in the beluga, *Huso huso*, which they explain by the feeding habits of this predator: benthos-eating species, for example *Rutilus rutilus caspius* and the species of the *Neogobius* genus, which are the second intermediate hosts of the nematode, significantly contribute to the nutrition of the predator. In *R. kutum*, larval stages of *E. excisus* were

confirmed by Mohammad *et al.* (2011), who specially pointed to the important problem of human infections, given that this fish species is frequently consumed in Iran and is habitually eaten raw.

In addition, *Eustrongylides excisus* larvae have been identified in various fish species in Turkey: the sand goby *Gobius fluviatilis* (Ozturk *et al.*, 2002), the big-scale sand smelt *Atherina boyeri* (Ozesen, 2011) and the bream *Abramis brama* (Karatoy & Soylu, 2006), in the round goby in the Baltic Sea (Kvach & Winkler, 2011). Although it has also been found to parasitize the pike-perch in the Caspian Sea (Movahed *et al.*, 2009), only limited data is available relating to the infection in this fish species.

According to Kostić and Maletin (1988) in Serbia pike-perch feeds on *Carassius gibelio*, *Rutilus rutilus* and *Blicca bjoerkna*, which are also intermediate hosts. Thus, there are favourable conditions for the pike-perch to be the reservoir of this zoonotic nematodosis, which is to be remembered. In Serbia, the pike-perch is a highly appreciated fish species, due to its tender and delicious meat and high protein content (Ćirković *et al.*, 2011) and is frequently consumed. The problem is that the pike-perch is frequently prepared on lower temperatures, taking less time in comparison with cyprinid fishes; thus, the consumers and food processors should be informed not to undercook the fish and enable the destruction of *E. excisus* larvae, which may possibly be present in the meat.

These findings represent the first observations of *Eustrongylides* larvae in pike-perch caught in Serbia. This constitutes a very important finding, because *Eustrongylides* larvae have a significant effect on their predatory fish hosts. According to Moravec (1994), the effects of the parasite infection include, but are not limited to, scars situated at the place of attachment of the larvae, kidney failure and inflammatory lesions on various organs such as the stomach and intestine. This was accounted for in our study, where the afore mentioned scars were registered. Further investigation and monitoring of *Eustrongylides* prevalence in pike-perch, but also fertility and abundance of this fish species should be conducted. These investigations may yield further results on the effects and impact of *Eustrongylides* larvae on pike-perch.

Conclusion

Larvae of the species of the genus *Eustrongylides*, as is *Eustrongylides excisus*, belong to the large group of food-borne parasites. Although it is common knowledge that the pike-perch is traditionally not consumed raw in Serbia, it is usually thermally processed on lower temperatures than are cyprinid fish species, thus rendering the possibility of survival of the larvae undoubted.

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