PRELIMINARY RECORDS ON THE PRESENCE OF THE NEMATODE *Eustrongylides excisus* AT THE FISH SPECIES *Silurus glanis* AND *Perca fluviatilis* FROM VICTORIA LAKE (BRATOVOIEŞTI - DOLJ)

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Abstract. The present paper brings together comments on a nematodosis characteristic at the fish species *Silurus glanis* and *Perca fluviatilis* from Victoria Lake. This is part of a lacustrine complex called "Adunaţii de Geormane", forming an isolated biological unit linked to the Jiu River by a channel used for the discharge of the water surplus through Marica pool (MARX et al., 1967). Are described the etiology and pathology of the disease, as well as prophylaxis and treatment measures mentioned in the specialized literature.

Keywords: nematodosis, *Eustrongylides excisus*, lacustrine complex.

Rezumat. Date preliminare privind prezenţa nematodului *Eustrongylides excisus* la speciile *Silurus glanis* şi *Perca fluviatilis* din Lacul Victoria (Bratovoieşti - Dolj). În acest material se prezintă o nematodoză întâlnită la peşti din speciile *Silurus glanis* şi *Perca fluviatilis* în Lacul Victoria. Acesta face parte din complexul lacustru „Adunaţii de Geormane", care formează o unitate biologică izolată, legată de Jiu printr-un canal de evacuare a surplusului de apă prin intermediul bălţii Marica (MARX et al., 1967). S-au descris etiologia şi patologia bolii, precum şi măsuri de profilaxie şi tratament cunoscute din literatura de specialitate.

Cuvinte cheie: nematodoză, *Eustrongylides excisus*, complex lacustru.

INTRODUCTION

Our research aimed at gathering the ichthyologic material necessary for further parasitological studies. The study site was represented by Victoria Lake, which, together with Marica Lake, form a lacustrine complex located on the left upper terrace of the Jiu (Figs. 1, 2, 3). Being located in the immediate proximity of Craiova city, this lacustrine complex used as one of the main tourist attraction spots for Dolj County in the ´70s. Consequently, intensive sportive fishing, as well as industrial fishing was practiced in the area, and riparian population benefitted from a valuable food resource (Figs. 4, 5). Huge amounts of fish were fished in these lakes, especially Asian carp; Victoria Lake was populated with this species in 1968. These phytophagous fish completely changed the appearance of the lake, as they were introduced for cleaning the water of the extremely large amounts of phytoplankton. An older study regarding the lacustrine complex was achieved in the period 1964 -1967; it dealt with different hydrobiological (MARX, 1964) and ichthyologic aspects (GOGA, 2005).

Figure 1. Study site – Victoria Lake (original).

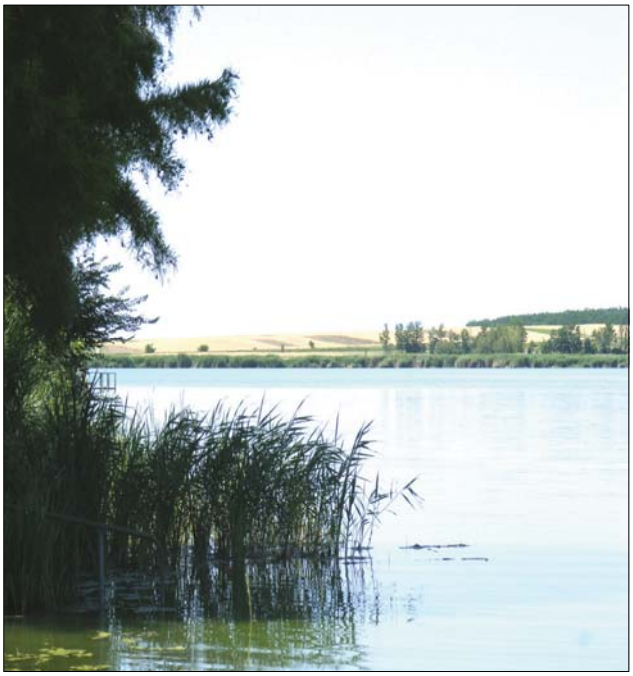

Figure 2. Lake Victoria (original).

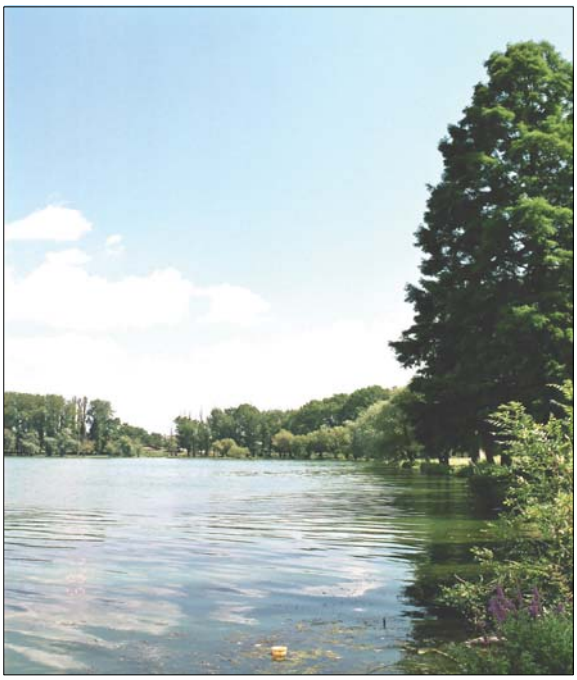

Figure 3. Lake Victoria (original).

Figure 4. Lake Victoria, a tourist attraction spot for Dolj County (original).

Figure 5. Lake Victoria, a tourist attraction spot for Dolj County (original).

The nematode worm genus *Eustrongylides* JÄGERSKIÖLD, 1909 is framed taxonomically in: Ph. Nematoda, Cls. Adenophorea, Ord. Enoplida, Fam. Dioctophymatidae (FAUNA EUROPAEA, 2013; NCBI Taxonomy, 2009; BioLib. cz. Import, 2009), but there are also different views according to which this family belongs to Cls. Secernentea, Ascaridida (EOL-ITIS, 2009) or Rhabditida (EUNIS, 2012) Orders. The most frequent species are: *tubifex, ignotus* and *excisus.* Their geographical distribution is as it follows: *E. excisus* – Europe, Russia, Middle East, India, Taiwan, Australia; *E. tubifex* – United States, Canada, Brazil, rarely in Europe (Russia); *E. ignotus* – United States, Brazil, New Zeeland. These three *Eustrongylides* species that determine the nematodosis of aquatic (mainly fish-eating) birds and prey-birds, as definitive hosts, present a similar life cycle, involving two or three intermediary hosts: an aquatic invertebrate and one or two wild and cultured fish species.

Recently, Chinese authors (XIONG et al., 2013) reported the first molecular phylogenetic analysis of larval *Eustrongylides* sp. collected from different fish species. It was also analysed their host specificity that presented in China low degree, but exhibited some host preferences.

Eustrongylides excisus JÄGERSKIÖLD 1909 is the etiologic agent of the parasitic disease called Eustrongylidosis at ichthyophagous and prey birds (COLE, 2013). This species has the widest geographic distribution (Eurasia - Australia), probably in connection with large circulation of intermediate and definitive hosts. It grows well in standing and less flowing water.

E. excisus larvae were identified, for the first time in the lacustrine complex Victoria – Marica (Dolj County, Romania), at two predator fish species: *Silurus glanis* (Ord. Siluriformes) and *Perca fluviatilis* (Ord. Perciformes), the last one being found in the trophic chain of the catfish.

MATERIAL AND METHODS

In April 2013, there were organized field trips in the area of the lacustrine complex Victoria – Marica, located at a distance of 25 km from Craiova, on Rojiştea terrace (COTEŢ, 1957); there were sampled by means of a monofilament net, 100 m long and 2 cm wide meshes. In order to investigate the incidence of the nematode larvae, there were studied the previously collected 20 fish specimens belonging to four species: *Perca fluviatilis*, *Abramis brama, Sander lucioperca* and *Silurus glanis*. The collected fish material was weighted, measured and examined from ichthyopatological point of view, namely clinic examination and parasitological examination in the Parasitology laboratory of Sanitary Veterinary Direction Dolj; the parasite was identified at only two fish species: the perch (*P. fluviatilis*) and the catfish (*S. glanis*). Referring strictly to the parasitological examination, we mention that it was achieved in order to identify endoparasites according to the usual methods, generally accepted in ichthyoparasitology (BOGATU & MUNTEANU, 2008). The identification of nematode larvae was made directly by means of macroscopic examination (Fig. 6), taking into account that larvae are 23 - 27 mm long, and by microscopic examination (fresh preparations microscopic slide-coverglass).

It was performed the fish necropsy, starting with an incision made with a pair of scissors along the abdomen. The entire abdominal cavity was exposed, searching possible pathological formations. The red parasite was placed on a Petri dish in distilled water and its visualization was made through transparency at Olympus SZX7 stereomicroscope with the objectives 2x, 3,2x; ocular WHSZ 10x/22 (Fig. 10), as well as at the optical microscope Olympus BX 43, as fresh preparations microscopic slide-coverglass, with the objective 10x; ocular WHN 10x/22 (Figs. 8, 9). After the examination, the parasites were fixed in 4% formaldehyde in a glass jar (Fig. 7).

Figure 6. Macroscopic visualisation of the parasite, from gills to anus, *Perca fluviatilis* and *Silurus glanis* (original).

Figure 7. 4% formaldehyde-fixed parasites (original).

RESULTS AND DISCUSSIONS

The nematode *Eustrongylides excisus* JÄGERSKIÖLD, 1909, was identified at perch (6 specimens) and catfish (100 specimens), both as encysted larvae in a thick capsule of conjunctive tissue (L4) (Fig. 8), on the mesentery or in intercostal and abdominal muscles, intestinal wall, liver, ovaries, as well as free larvae in the abdominal cavity or non-encysted larvae in the muscles of the abdominal wall (L3) (Figs. 11, 12). The two predator fish species are considered second-stage intermediate hosts, storage the third and fourth stage larvae. Fish are important in the spread of parasite in ichthyophagous bird populations.

Larvae are large, bright red, 11 – 27 mm (some data indicate even 83 mm) long and a diameter of 2 mm, surrounded in some cases by a thick brown capsule (cysts).

The data published in the specialized literature emphasize that the life cycle of *Eustrongylides* spp. includes 4 larval development stages, interposed between the egg and the adult phase (VULPE, 2007; COLE, 2013; CRUCERU, 2013). Definitive bird host feed on infected fish and then nematode larvae develop to sexual maturity. The eggs laid by adult nematode worms in the body of the definitive host (in our case: little egrets, herons, cormorants), are released into the aquatic environment together with the feces of the bird. First stage larvae (L1) develop in eggs ingested by an oligochaete worm of the genus *Tubifex* (first intermediate host). Eggs hatch within oligochaetes, releasing (L1) and second an third - stages larvae (L2 and L3) develop in oligochaetes too; third-stage larvae preserves the cuticle of the first and second molt (MEASURES, 1988). Minnows, juvenile and other small fish (for example *Fundulus* and *Gambusia*), as second intermediate host, can feed upon oligochaetes that so transfer L3 to these fish.

The two fish species (perch and catfish), that the *Eustrongylides excisus* larvae has been identified in Marica and Victoria Lakes, feed on these oligochaetes and thus the third stage larvae (L3) become encapsulated within fish body; on the internal surface areas of the fish the nematode larvae develop into the infective fourth larval stage (L4), awaiting to be ingested by fish-eating birds. Small fish were also found in the stomach of larger parasitized fish and we deduced that they might be facultative second intermediate hosts for this parasite.

Finally, these will be eaten by ichthyophagous birds as definitive hosts (Ardeidae, Ciconiidae, Plataleidae, Pelicanidae, Podicipedidae, Gavidae, Phalacrocoracidae) (CRUCERU, 2013), the infestation taking place during the entire year. Also happens that infested minnows or other small fish are feed upon by species of other than birds, as: predator larger fish, amphibians and reptiles (snails, crocodiles). They are facultative second - stage intermediate hosts or paratene (transport) hosts and there larvae (L3) develop to (L4) that become encysted. So, SLOBODA et al. (2010), reported in Sinoe Lake communicating with the Black Sea (Histria, Romania), the occurrence of *Eustrongylides excisus* larvae (subcutaneous L4 in granulomatous lesions) in snakes *Natrix tessellata*, as paratene hosts, by 3 species of *Neogobius* (gobiid fish, Ponto-Caspian relict) infested with *E. excisus* consumption. The authors concluded that this nematode parasite in Gobiidae has limited influence on its fish-host susceptibility to predation by snakes. An interesting observation was published by the Russian authors (MIKAILOW et al., 1992) which found eggs of *E. excisus* in sturgeons from the Caspian Sea. They expected that under certain conditions sturgeons can be definitive host for this parasite nematode. Remember that Delta fishermen have reported also this in sturgeons (INFOPESCAR.tv, 2010; CRUCERU, 2013).

The symptoms characteristic to this nematodosis are not known in case of fish. It is presumed that there occur certain dysfunctions in their growth process and difficulties in reproduction at the severely affected individuals, at which cysts are developed in the digestive or sexual organs. High intensity of infestation with nematode larvae could cause symptoms of disease in the fish organism: this becomes lethargic, with swollen abdomen and difficulty in swimming (INFOPESCAR.tv, 2011).

Eustrongylidosis is characteristic in aquatic birds (mainly fish-eating) or prey-birds, as definitive hosts. These consume fish, frogs, snails etc., swallowing (L4) larvae, that become adults and reproduce. Adult worms are reddish up to 151 mm in length and 4.3 mm in width ("wire worm") and dig galleries in the digestive wall muscles. In heavy infestations large mortalities register in young birds; especially nestling die-off in coastal rookeries, in particular of egrets and other wading birds (COLE, 2013; INFOPESCAR.tv, 2011).

In man Eustrongylidosis is extremely rare, there have been only 5 cases reported in U.S. (U.S. FDA, 2013). If the larvae (L4) are eaten in undercooked or raw fish, they can attach to the wall of the digestive tract, in muscles, forming granulomas in the stomach and intestine. Clinical symptoms have been reported: the parasite that perforates the gut wall causes verminous peritonitis and the migration of *E. excisus* larvae to other organs are accompanied by severe abdominal pain. CRUCERU (2013) estimates that there could be dozen of cases in the world, caused by eating fish, pike roe possibly.

In Romania*, E. excisus* was first signalled by CIUREA who, during 1910-1928, identified it in the muscles of barbel, pike, perch and other fish species in the Danube Delta (ROMAN, 1955). Much later it was mentioned by (VASILIU & RĂDULESCU, 1946), at the catfish from the Danube Delta, while RĂDULESCU (1947, 1948) identified it at perch in Fundeni Lake near Bucharest; during the last ten years there were reported also zander and catfish parasitized by *E. excisus* larvae in some ponds around Bucharest (INFOPESCAR.tv, 2011). In the lower basin of the Danube, ROMAN (1955) in her PhD Thesis reported larvae of *E. excisus* encysted in liver and intestine (L4), at these hosts, indicating the prevalence of the infection: asp (60%), bleak (20%), catfish (10%), pike (5%), perch (25%; with 1-4 individuals / host).

Over 20 years there were found larvae of *E. excisus* encysted in the abdominal muscles, intestinal wall, body cavity or liver and gonads, in the most predatory fish species (perch, pike, zander, catfish, asp) in the Danube Delta (especially Roşu and Roşuleţ Lakes, Musura bay) (www.infopescar.tv, 2010-2011). More recently this was reported in fish from the Danube coves (Oltenia); currently, it is spread throughout the country and its prevalence is increasing). The most affected species is *Perca fluviatilis* (10-15 larvae / fish). During the last 2 decades in different places in Romania there were found also *E. excisus* larvae in Asian herbivorous fish species (the grass carp mainly) acclimatisated from China (CRUCERU, 2013). The parasite was also identified in the basins of the Preajba Valley River (Dolj County), at perch and zander, both as larva enclosed in a thick capsule of conjunctive tissue on the mesentery and ovaries and as a free larva in the abdominal cavity and in the muscles of the abdominal wall (GOGA, 2009).

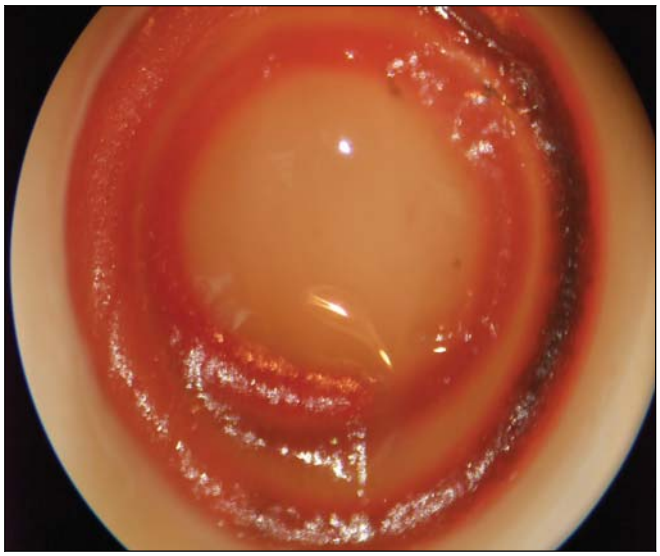

Figure 8. Larva encysted in a thick capsule of conjunctive tissue.

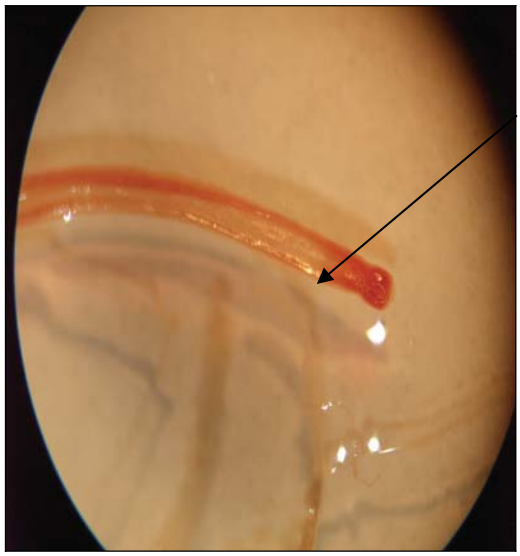

Figure 9. Anterior extremity of the larva, visualization at Olympus BX 43 microscope, as native preparation microscopic slide-coverglass, objective 10x; ocular WHN 10x/22 (original).

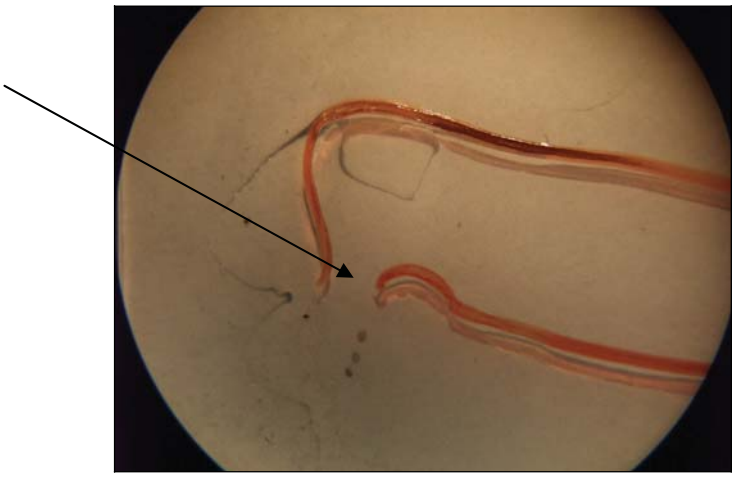

Figure 10. Posterior extremity of the larvae, visualisation through transparency at Olympus SZX7 stereomicroscope, objectives 2x, 3,2x; ocular WHSZ 10x/22 (original).

Figure 11. Free larva in the abdominal cavity *Silurus glanis* (original).

Figure 12. The parasite penetrates the muscles of the abdominal wall *Silurus glanis* (original).

E. excisus larvae were found also in some species of gobiid fishes (Perciformes), Ponto-Caspian relict, in the Middle Danube (Slovakia) at bighead goby (*Neogobius* (*Ponticola*) (*kessleri*) (ONDRAČKOVA, 2009) and since 2005 it has been reported at monkey goby (*Neogobius fluviatilis*) in Ukraine (the Dniester River estuary, NW Black Sea) (KVACH, 2005). In Poland (the lower Vistula River region), there were detected in a significant proportion larvae of *E. excisus* (L.) parasites at the Chinese sleeper *Perccottus glenii* (Ord. Perciformes, Gobioidei) as intermediate host (MIERZEJEWSKA et al., 2010). This freshwater fish species originates from Eastern Asia (Amur, China, Korea) and is invasive in N-Eurasia (Russia, Belarus, Ukraine); it was colonized in Poland during the last two decades mainly in the Vistula River basin, probably connected with the Asian herbivorous fish species. All in Poland, at ornamental fish species *Symphysodon aequifasciatus* (wild blue discus) (Ord. Perciformes, Cochlidae) imported from Brazil (the Amazon region), *Eustrongylides* sp. larvae were reported for the first time (SOBECKA et al., 2010). At *Silurus glanis* from Aras Dam Lake (Iran) MASSOUMIAN et al. (2006) reported cysts of *E. excisus* located in the body cavity, attached to the external part of the intestine. It will be interesting as more data obtained in different European countries to be compared with those in East Asia and possible to prove the origin of their status of native (indigenous) or invasive (non-indigenous) species of the parasite nematode *E. excisus*. So, *E. excisus* larvae were found in the Danube, the Visla, the Dniepr basins or the Black Sea, both in native and invasive fish species. In the Lower Danube (Romania) it was noted the preference of this nematode worm for Perciformes (perch, zander, Gobioidei) and Siluridae fish, perhaps part of second-stage intermediate hosts, as voracious predator (www.infopescar.tv, 2011). Analysing the host specificity for *Eustrongylides excisus* at the molecular level in different fish species in China, XIONG et al. (2013) found some host preference, the most obvious being at Perciformes (Gobioidei) and Asian swamp eel *Monopterus albus* (Ord. Synbranchiformes) originating in the water of East and Southeast Asia; it was also found at a *Silurus* species.

CONCLUSIONS

The present paper reports for the first time the occurrence of *Eustrongylides excisus* JÄGERSKIÖLD, 1909 infestation in some lakes for the middle course of the Jiu River (Dolj County).

The data published so far referring to the larvae of the nematode *E. excisus* on different fish species in Romania support the status of native species of this parasite in the middle course and lower basin of the Danube, as well as in the north-western Black Sea; moreover, some hosts (*Neogobius* sp., Gobiidae, Perciformes) are Ponto-Caspian relict (KVACH, 2005; ONDRAČKOVA et al., 2009; SLOBODA et al., 2010), such as the sturgeon from the Black and the Caspian seas (MIKAILOV et al., 1992; www.infopescar.tv, 2010; CRUCERU, 2013). It will be interesting to closely detailed on its presence in non-indigenous (invasive, acclimatized) fish species.

Among fish hosts of *E. excisus* larvae, in the lower basin of the Danube (Romania) the highest prevalence and intensivity of infestation was found at Perciformes, especially in *Perca fluviatilis*.

It is well-known that the eutrophication phenomenon and warm water temperatures (20-30^{o}C) creates optimum conditions for the numerical increase of the aquatic oligochaete, as well as of fish and fish-eating birds populations. Thus, water quality is a determinant factor for the transmission of the parasites (COLE, 2013). So, it was showed that water eutrophication by pollution (the Danube Delta, the Black Sea and littoral lakes) favoured the emergence of infestation with nematode *E. s excisus*, as well as the increasing of its prevalence (intensivity) in oligochaete, gobiid and cormorant populations (SLOBODA et al., 2010).

The improvement of the water quality should be taken into account as a prevention means in case of infestation with *Eustrongylides*, as well as the reduction of the amounts of fertilizers and of the discharged residual water (from the household located on the left shore of the lake), as all these have a negative impact on the ichtyofauna. An explanation would be the presence of ichthyophagous birds during that period compared to that of winter, as well as the fact that water temperature increased and the content of organic substances generated by the decomposing aquatic macrophytes thickens the silt layer and increases the quantity of toxic organic substance present in the water.

The prevention of Eustrongylidosis is not possible presently, but, in natural environments, it got rarer and it does not induce obvious pathological states; however, it reduces the commercial value and meat quality. In order to fight against these parasitosis there were proposed a series of prophylaxis and treatment measures, such as the limitation of the access of ichthyophagous birds by non-creating their favourable biotope and periodical draining.

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