PRELIMINARY DATA ON THE INFESTATION OF THE FISH SPECIES *Perca fluviatilis* L., 1758 BY *Diphyllobothrium latum* (LINNAEUS, 1758) (CESTODA. PSEUDOPHYLLIDEA)

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Abstract. The paper presents the preliminary results on the parasitizing of the fish species *Perca fluviatilis* L., 1758 by the cestode *Diphyllobothrium latum* plerocercoid larvae identified only once, in April 2011, in the gut of three specimens of perch from the small reservoirs located on the Preajba river, a small tributary of the Jiu River. The parasite was identified by viewing in dark field illumination by means of stereomicroscope, as well as by means of the optical microscope, as a fresh preparation slide-coverglass.

Keywords: cestode, *Diphyllobothrium latum*, plerocercoid larvae, small reservoirs.

Rezumat. Date preliminare privind infestarea speciei *Perca fluviatilis* L., 1758 cu *Diphyllobothrium latum* (Cestoda. Pseudophyllidea). În lucrare sunt prezentate rezultatele preliminare privind parazitarea speciei *Perca fluviatilis* L., 1758 cu larve plerocercoide ale cestodului *Diphyllobothrium latum*, identificat în luna aprilie a anului 2011, o singură dată, în intestin la trei exemplare de biban în lacurile mici de baraj de pe cursul râului Preajba, un mic afluent al Jiului. Evidenţierea parazitului şi identificarea lui s-au realizat, prin vizualizarea în lumină reflectată pe un fond întunecat la stereomicroscop, dar şi la microscopul optic, ca preparat nativ lamă-lamelă.

Cuvinte cheie: cestod, *Diphyllobothrium latum*, larve plerocercoide, lacuri mici de baraj.

INTRODUCTION

Cestode tapeworms are exclusively parasitic, having an elongated body, typically segmented and extremely dorso-ventral flattened (like a ribbon). Generally, the adults inhabit the intestines of their final hosts, being anchored to the intestinal wall by, of type specific, holdfast organs (scolex at the anterior end). The ontogenesis proceeds as metamorphosis employing different larval stages. As larvae, they parasitize both vertebrates and invertebrates, the life cycle involving one or two (possibly three) intermediary hosts.

Pseudophyllidean cestodes are attached to the intestinal wall with two longitudinal bothria.

Diphyllobothrium Cobbod, 1858 (Ph. Platyhelminthes, Cl. Cestoda, Subcl. Eucestoda, Ord. Pseudophyllidea, Fam. Diphyllobothriidae) is a genus of tapeworm, which can cause Diphyllobothriosis in man, through consumption of raw or undercooked fish. Due to the increasing popularity of such a nutrition manner, numerous cases of human infection have appeared recently even in developed countries (JACKSON et al., 2007; SCHOLZ et al., 2009).

There are known more than 50 valid species of *Diphyllobothrium* and 14 spp. of them have been reported from humans. Adult tapeworms may infest humans, canids, felines, bears, pinnipeds, mustelids and piscivorous birds (final hosts); plerocercoid larvae (infective stage for the final host) can be found in small freshwater fish (minnows and others), which are ingested by large predator fish (second intermediate hosts). Most common intermediate hosts, especially of *Diphyllobothrium latum*, are brackish and freshwater predatory fish (such as *Perca fluviatilis*, *Esox lucius*, *Lota lota*, *Acerina cernua*) in Europe (MUNTEANU & BOGATU, 2008; SCHOLZ et al., 2009) and *Sander canadensis* in North America (ANDERSON & GIBSON, 1989). Plerocercoids differ from each other in the body surface (wrinkled or smooth), the length of microtriches, the retraction of scolex and the number of subtegumental longitudinal muscles (ANDERSON & GIBSON, 1989). MUNTEANU and BOGATU (2008) considered as differential criteria: shape and size of the plerocercoid larvae, the composition of its cephalic end, location in the fish body, host species. *D. latum* plerocercoids have often been identified in salmonids (salmon, trout, whitefish) but this identificationis is questionable (SCHOLZ et al., 2009). Plerocercoids develop rapidly into adults in the final host's intestine.

Diphyllobothrium latum (Linnaeus, 1758) has a high pathogenicity (ROMAN, 1955) and it is wide spread in Northern Hemisphere (Europe, West Russia, North America), Asia and South America. *D. latum* has been considered to be the main species infecting humans in Europe, whereas *Diphyllobothrium dendriticum* is present in Northern Europe (DUPOUY-CAMET & PEDUZZI, 2004). The carrying of some animals constituting intermediate hosts of different parasites have led in many cases to its expansion and so *Diphyllobothrium latum* reached Chile with rainbow trout (*Salmo gairdneri*) from North America and lake trout *Salmo trutta lacustris* from Europe (MEHLHORN, 1988).

Diphyllobothriosis is the most important fish-borne zoonosis and main pathogens are *Diphyllobothrium latum* and *Diphyllobothrium nikonkaiense* (in Japan and Korea, SONG et al., 2014); the latter species was carried out molecular studies (ARIZONO et al., 2009; SCHOLZ et al., 2009). Up to 20 million humans are estimated to be infected worldwide. In some countries (Russia, South Korea, Japan, Brazil, alpine lakes in Switzerland, Northern Italy and Eastern France (JACKSON et al., 2007; SCHOLZ et al., 2009; SONG et al., 2014) diphyllobothriosis has shown a reemergence.

Larval Diphyllobothriosis (plerocercoidosis) occurs in predator fish (the 2nd intermediate host) as pike, perch, burbot, ruff.

MATERIAL AND METHODS

The research for obtaining the ichthyologic material necessary to the parasitological studies was conducted on the small reservoirs located along the Preajba river, a small tributary of the Jiu and they began in 2009 (GOGA, 2009; GOGA & TÎMBURESCU, 2011; GOGA & CODREANU BĂLCESCU, 2011; GOGA, 2012; GOGA & TÎMBURESCU, 2012a; GOGA & CODREANU BĂLCESCU, 2013; GOGA & TÎMBURESCU, 2013a); the parasite was identified in April 2011 at perch (*Perca fluviatilis*). In order to take samples, it was used a monofilament fishing net with a length of 100 m and a mesh size of 4.5 cm, and a pneumatic boat.

The diagnosis of the parasite was established after the necropsy of the suspected specimens of perch, performed (Fig. 2) through an incision along the abdomen, from the gills to the anus, using scissors. The entire abdominal cavity was exposed, following any pathological formations. Highlighted plerocercoid larvae were removed from the intestines (Figs. 2a, 3) and viewed at the optical microscope and stereomicroscope, and, then, stored in a special plastic container in 4% formaldehyde.

Figure 1. Fish weighted before being gutted.

The cestode larval stages were emphasized and identified after washing it with distilled water and viewing it through transparent incident and reflected light against a dark field. Such observations were performed at the stereomicroscope Olympus SZX7 with the objectives 2x, 3.2x; ocular WHSZ 10x/22 (Figs. 4, 4a), as well as examination at the optical microscope Olympus BX 43, as fresh preparation slide – coverglass, with the objective 10x; ocular WHN 10x/22 (Fig. 5).

RESULTS AND DISCUSSIONS

Diphyllobothrium latum larvae sampled from predatory fish species have been reported in Romania at pike (ANTIPA, 1909; IAMANDI, 1936, Jijia) after V. BABEŞ (1853) identified *Esox lucius* as intermediate host for *D. latum*. These data were mentioned by Elena ROMAN (1955) who found herself this parasite in one specimen of perch in the Danube Delta (Mila 23). V. VULPE (2007) also reported *D. latum* from Greaca settlement.

Figure 2. Parasite (plerocercoid larva) removed from the perch intestine.

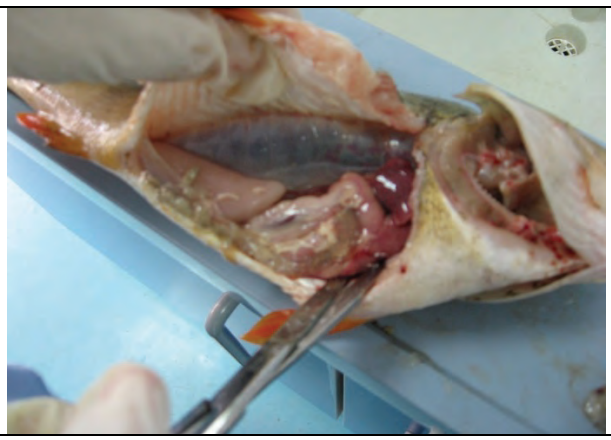

Figure 2a. The necropsy of the fish using scissors.

Figure 3. Removal of the plerocercoid larva from the parasitized fish intestine.

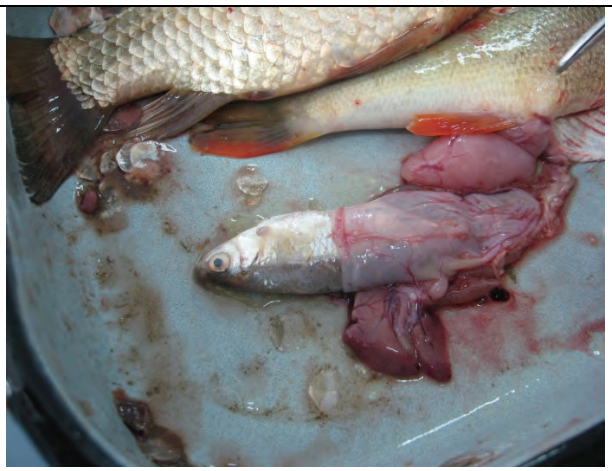

Figure 3a. Ingested fish (*Pseudorasbora parva*) identified in the stomach of the perch, possible intermediate host for plerocercoid larvae.

There were found some eucestode larvae, only once, in the intestines of three specimens of perch (average length=27 cm; average width=9,5 cm) weighing between 272 g and 368 g (Fig. 1). According to their morphological features, we could determine the belonging of highlighted cestode larval stages to the genus *Diphyllobothrium*, respectively *D. latum*, as the second larval stage – plerocercoid (sparganum).

Plerocercoid larva has an elongated body (2-3 mm up to 5-6 cm in length; 1.2-3 mm in width). It can be seen an oblong cephalic area, not separated from the rest of the body, and provided with two bothridial grooves more or less prominent; the cephalic end is undifferentiated (Figs. 4, 4a). Larva is dirty white or cream, slightly transparent and the surface is wrinkled transversely.

Plerocercoid larvae parasitize fish body, in almost any organ (liver, gonads etc.) and frequently even free in the abdominal cavity. The sites may differ according to the fish species and plerocercoids from viscera may migrate to the body wall musculature, after the death of the host. Usually they lie unencapsulated (MUNTEANU & BOGATU, 2008; SCHOLZ et al., 2009).

We have found plerocercoid larvae localized in the gut of the three specimens of perch (Figs. 2a, 3).

One of the parasitized perch submitted to dissection, had into the stomach contents, a small fish (7 cm long and 3 cm wide) identified as *Pseudorasbora parva* (Ord. Cypriniformes, Fam. Cyprinidae) (Fig. 3a). It is an invasive species of East Asian origin that entered Europe (the Dniester, the Dnieper and the Danube basins) in the last 50 years. They abound now in stagnant freshwater, lowland and hilly, and have meat diet, feeding on aquatic invertebrates, eggs or fry of other fish species. We think it is possible in this case, that the carnivorous fish *Pseudorasbora parva* to interpose in the life cycle of *Diphyllobothrium latum*, as the second intermediate host (2nd I. H.) (like paratenic host) and after, the predator fish *Perca fluviatilis* to be a 3rd intermediate host (3rd I. H.). Both fish hosts are parasitized by plerocercoid larvae of *D. latum* (sparganum) (MEHLHORN, 1988; SCHOLTZ et al., 2009).

In the life cycle of *Diphyllobothrium latum*, adult cestodes living in the intestine of final host eliminate the operculated, unembryonated eggs in the water with the host faeces. Completion of the development to coracidium larvae takes one to several weeks, depending on the water temperature.

As a parasite of the temperate latitudes in the Northern Hemisphere, *D. latum* is adapted to lower temperatures. The embryonic development is optimum at a water temperature of 18-20^{0}C (in correlation with the high oxygen demand). Also, as in the free-living stages, the developmental speed of parasites in invertebrate intermediate hosts is temperature dependent (MEHLHORN, 1988).

Free ciliated coracidium larval stages, containing hexacanth oncosphere, emerge having been ingested by several species of zooplanktonic copepod crustaceans, within which the development of the second-stage larvae occurs (in general body cavity). Approximately 40 spp. of copepod crustaceans genera (Diaptomidae and Cyclopidae) serve as the first intermediate hosts (TORRES et al., 2007). Olivia CIOBOIU (2002) cites in planktonic restricted populations existing in small dam reservoirs on the river Preajba (Dolj County) 5 spp of Cyclopidae: *Acanthocyclops vernalis, A. viridis, Eucyclops macruroides, E. serrulatus, Mesocyclops crassus*.

Diphyllobothrium latum could become endemic if in certain conditions (for example a dam construction in the area of a river) copepods increase above a critical density (MEHLHORN, 1988).

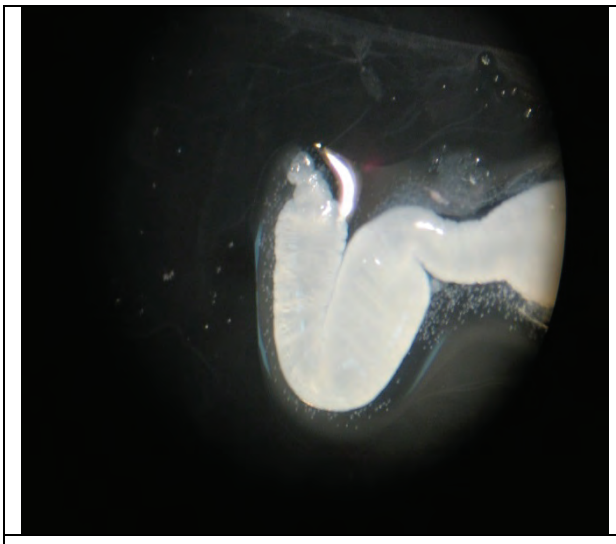

Figure 4.

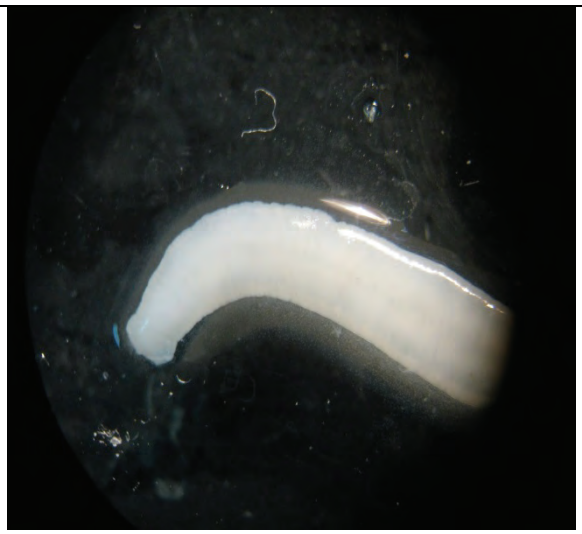

Figure 4a.

Highlighting the parasite (plerocercoid larvae) at stereomicroscope**.**

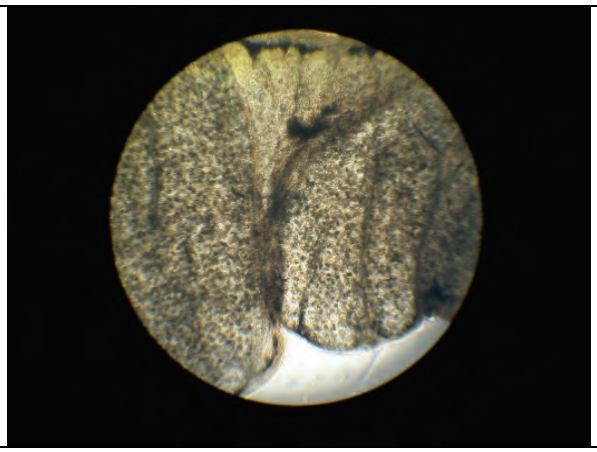

Figure 5. View at the optical microscope Olympus BX 43 of the fresh preparation slide-coverglass: subtegumental longitudinal muscles of plerocercoid larvae (objective 10x; ocular WHN 10x/22).

As second intermediate hosts predator brackish and freshwater fish become infected by ingesting infect copepods. Inside their intestine, the procercoid is released in the body cavity and muscles, where it grows rapidly into a plerocercoid (sparganum), which remains mainly undifferentiated.

In *D. latum*, plerocercoids may accumulated without further development, in the muscles (not encysted) of carnivorous fish (paratenic hosts) (MEHLHORN, 1988; SCHOLTZ et al., 2009).

Infestation of final hosts (humans, fish eating animals: mammals – cats, dogs etc.; piscivorous birds) occur by ingestion of raw meat of fish containing plerocercoids. Having reached the final host intestine, the plerocercoids grow rapidly and become adult worms in 5-6 weeks, being attached to the intestinal wall with 2 longitudinal bothria. *Diphyllobothrium latum* reaches a maximum size of 25 m (http://wikipedia.org/wiki/Diphyllobothrium. 30 April 2014).

In pathogenic terms, larval Diphyllobothriosis is less dangerous to fish and generally specimens infested with *Diphyllobothrium latum* plerocercoid larvae do not show obvious signs of parasitation. In heavy infestations, mentioned in the literature the growth rates slowed and there is a great danger for people when they consume fish hosting the plerocercoids (MUNTEANU & BOGATU, 2008). In our case, the parasite plerocercoid was identified accidentally and the three specimens of perch were parasited only by one larva.

As prophylaxis literature recommends limiting of Diphyllobothriosis extension by: detection of outbreaks, interrupting the parasite life cycle (for example combating copepods), spill prevention of dejections in water, supply basins with clean water, health education of the population in order to correct fish eating. Water treatment with Trichlorfon (0.2 mg / l) and with calcium hypochlorite (500-600 kg/ha) did not give satisfactory results.

CONCLUSIONS

The plerocercoid larvae of the cestode tapeworm *Diphyllobothrium latum* were identified in predatory fish species *Perca fluviatilis*, as the second intermediate host in the life cycle of this parasite, in full accordance with the data reported in the literature from other sites in Romania, as in other European countries. Copepode crustaceans (Cyclopidae) constitute probably the first intermediate host.

The sources of plerocercoid larvae (infective stage) are the sick and old fish hosts, the parasite being not present at the species with a benthic feeding. *Diphyllobothrium latum* plerocercoid larvae were detected only in the spring season, when water temperature is lower; it is assumed that young receptive host fish feed on the 1st intermediate hosts of this parasite (planktonic copepods) or small fresh water fish, as *Pseudorasbora parva*.

In future research it will be interesting to expand surveillance for some planktonic copepods and predator fish species to find parasite larvae of *Diphyllobothrium latum* in the same environmental conditions, as well as prospecting of possible cases with human Diphyllobothriosis in this region.

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