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Original Article
Özgün Araştırma

Morphological identification and pathology of *Myxobolus cyprini* and *Lamproglena pulchella* in some fish in Turkey

Aysel İtik Ekinci*[1], Naim Sağlam[2], Mustafa Özkaraca[3], Ünal Kılınç[4]

[1,4] *Veterinary Control Institute Directorate, Elazig, Türkiye*
[2] *Fırat University, Faculty of Fisheries, Department of Aquaculture and Fish Diseases, Elazig, Türkiye*
[3] *Department of Pathology, Faculty of Veterinary Medicine, Cumhuriyet University, Sivas, Türkiye*

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Abstract: *This study was performed to investigate the parasites and pathology in the three different fish species, Barbus lacerta, Capoeta trutta and C. umbla, caught in wildlife in Turkey. The fish caught for this study were transported to the laboratory under ethical rules and investigated for parasites. According to the results, two different parasite species (Myxobolus cyprini and Lamproglena pulchella) were morphologically identified. Myxobolus cyprini was found in the soft tissue of the pectoral fins of C. trutta as an off-white, oval cyst. In the histopathological examinations of the cysts, the spores of M. cyprini were observed. Lamproglena pulchella* was seen in the gills of *B. lacerta*. No parasite was also encountered in *C. umbla*. These parasite species are the first records for wildlife fishes of Uzuncayır Dam Lake of Turkey.

Keywords: Fish, parasite, *Myxobolus cyprini, Lamproglena pulchella ,Turkey*

Türkiye'deki Bazı Balıklarda *Myxobolus cyprini* ve *Lamproglena pulchella* türlerinin'nin morfolojik identifikasyonu ve Patolojisi

Özet: Bu çalışma, Türkiye'de yaban hayatında avlanan üç farklı balık türü olan *Barbus lacerta, Capoeta trutta* ve *C. umbla*'daki parazitleri ve patolojisini araştırmak amacıyla yapılmıştır. Çalışma için avlanan balıklar etik kurallara uygun bir şekilde laboratuvara taşınmış ve parazitler yönünden incelenmiştir. Araştırma sonucunda iki farklı parazit olarak *Myxobolus cyprini* ve *Lamproglena pulchella* morfolojik olarak teşhis edilmiştir. *Myxobolus cyprini türü Capoeta trutta*'nın pektoral yüzgecinin yumuşak dokusunda kirli beyaz renkte, ovalimsi kist içinde olduğu tespit edilmiştir. Kistlerden yapılan histopatolojik incelemelerde *M. cyprini*'nin sporları gözlenmiştir. *Lamproglena pulchella* türü ise *B. lacerta*'nın solungaçlarında görülmüştür. *Capoeta umbla*'da herhangi bir parazite rastlanmamıştır. Bu parazit türleri Uzunçayır Baraj Gölü için ilk kayıt niteliği taşımaktadır.

Anahtar kelimeler: Balık, parazit, *Myxobolus cyprini, Lamproglena pulchella, Türkiye*

Introduction

The protein requirement in the world is met mainly from animal aquaculture. It has been emphasized that countries should try to meet the current protein deficit, which is predicted to increase together with the population growth, and aquaculture (Quaas et al. 2016).

Fish face many disease factors in their habitat. Parasites are one of such disease sources. Some of the essential parasite groups abounding most on fish are protozoa and copepods. While protozoa are parasites, which live in both the internal and external organs of fish, copepods are located in the outer parts of fish (Ekingen 1983; Hoffman 1999). The parasites can cause collectively damage to the fish as well as the organs in which they have localized. Due to the destruction the parasite causes, the appearance of the fish spoils, their nutritional value decreases, and their growth, and reproduction are prevented. Because of these effects of parasites, the demand of consumers for fish decreases. Owing to these reasons, the studies on the determination of the parasite fauna of fish in their habitats are of vital importance to develop protection and struggle methods (Barber et al. 2000; Williams and Jones 1994).

Myxobolus species are common in freshwater and marine fish. *Myxobolus cyprini* clinically reveals blood build-up in the kidneys, anemia, hyperemia, loss of fish scale, exophthalmos, ascites, and inflam-

Yazışma adresi / Correspondence: *Aysel İtik Ekinci, Veterinary Control Institute Directorate, Elazig, Türkiye*
e-posta: ayselitik@hotmail.com
** Herhangi bir kuruluştan maddi destek alınmamıştır.*
ORCID IDs of the authors: *[1]0000-0002-2730-7657 • [2]0000-0002-3163-8432 • [3]0000-0002-6359-6249 • [4]0000-0002-4859-4384*

mation (Molnar and Kovacs-Gayer, 1985). The development of *M. cyprini* parasite in the muscle of Carp has been investigated and reported by Dykova and Lom (1984). Molnar and Kovacs-Gayer (1985) detected *M. cyprini* on the liver, spleen, kidney, gill, intestine, skin, and muscle of *Cyprinus carpio*. Moreover, the cysts of *M. cyprini* were determined on fish species such as *Alburnus alburnus*, *Abramis brama*, *Blicca bjoerkna*, *Scardinius erythrophthalmus, Rhodeus sericeusamarus* (Molnar et al. 2002), *Rutilus rutilus, Tinca tinca* (Kirjušina and Vismanis 2007; Landsberg and Lom 1991) and *Leuciscus cephalus* (Holzer and Schachner 2001).

In the Turkey, myxosporean parasite fauna of some wild freshwater fish (*Capoeta tinca, Condrostoma angorense, Scardinius erythrophthalmus*) had been investigated, and *M .anatolicus, M. karaeri*, *M. cakmaki, M. samsunensis*, *M. ekingeni, M.scardinii, M. arrabonensis,* and *M. polati* have beens raported (Pekmezci et al. 2014; 2015; 2021a,b).

Lamproglena pulchella were found in the gills of the *Capoeta trutta* and *Chondrostoma regium* caught in Keban Dam Lake, and their morphological structure was examined by Saglam (1998). The population of *L. pulchella* present in the fish belonging to the Carp family caught from different pollution levels was researched (Galli et al. 2001a). It was also tried to determine the effect of water quality on the *L. pulchella* frequency of presence in *Leuciscus cephalus* (Galli et al. 2001b). *L. pulchella* was detected in the *C. nasus* (Jirsa et al. 2011; Jirsa et al. 2006) and *L. cephalus* (Jirsa et al. 2006) fish species caught in rivers in Austria. The parasite mentioned above was also determined in the gills of *L. cephalus* in Serbia (Cakic et al. 1998) and *Scardinius erythrophthalmus* in Turkey (Kus and Soylu 2013; Soylu 2012).

This study intended to determine the parasites in three different fish species (*B. lacerta, C. trutta* and *C. umbla*) that live in Uzunçayır Dam Lake (Turkey) and thus help identify the parasitic diseases which may cause fish deaths and economic losses in the fisheries farms.

Material and Method

Study location

Fish samples used in the study were obtained from Uzunçayır Dam Lake. This dam lake, has constructed on Munzur Stream in Tunceli city center in Türkiye between the years of 1996-2003 and has made with clay-core filled with sand and pebble. The height of the dam lake is 70 m, and it has 13.43 km^2 of surface area and 308 hm^3 of water volume (Anonymous 2014).

Fish samples

Fish samples and parasitological study

The present study was carried out on wildlife freshwater fish (*Barbus lacerta, Capoeta trutta* and *Capoeta umbla*) samples caught using gill nets in Uzunçayır Dam Lake of Turkey between December 2012 and March 2013. Fish were transported alive in tanks to the parasitology laboratory of Elazig Veterinary Control Institute. They were narcotized with an anesthetic substance within the framework of ethical rules. This study has been approved by the ethics committee of Elazig Veterinary Control Institute of the Republic of Türkiye, Ministry of Agriculture and Forest. Then fish were necropsied as systemic to examine skin, fins, gills, eyes, brain, muscle, heart, intestine, liver, gall bladder, spleen, stomach, intestine, gonad, and kidneys (Roberts 2012). During the systemic parasitological examination, the aforementioned tissues and organs of the fish were put into petri plates that included 0.9% of physiological water. Then they were examined firstly under a dissecting stereomicroscope (×2-×5) and later under a light microscope (×10 - ×100), and smears from some tissues were prepared.

Histopathologic study

The histological methods of Roberts et al. (2012) were used to prepare the organs and tissues of the fish for histopathological analysis. The fish organs and tissues that were determined parasites during the parasitological examination were fixed in 10% of buffered formaldehyde solution for histopathological examination. After the fixation, routine histopathological procedures were followed, such as dehydration, cleaning, paraffin infiltration, blocking, cutting and splitting, painting and assembly. The tissues put up to paraffin blocks was cut in 5-micron thickness with a microtome. Then the samples prepared with pathologic process were stained with Hematoxylin-Eosin and examined with a light microscope (×40-×100).

Microscopy and data analysis

Parasitological examination of fish were made under stereo microscope. Photographs of *Lamproglena pulchella* were displayed by using an SC50 camera and CS Olympus cellSens Imaging Software. Photographs and analyses of pathological data of *Myxobolus cyprini* were performed in Olympus B×53 light

microscope with image capture in a DP72 camera and morphometry using the DP2-BSW (ver.2.1) software (Olympus).

Species identification of the collected parasites after their fixation and preparation, was performed according to Bruno et al. (2006), Bychovskaya-Povlovskaya et al. (1964), and Lom and Arthur (1989). The prevalence of parasites on hosts was determined by the calculation method of Bush et al. (1997).

Ethics statement

Permission was obtained from the Elazığ Veterinary Control Institute Directorate Animal Experiments Local Ethics Committee (Approval number: 19.10.2012-10/1).

Results As a result of the study, two different parasite species *Myxobolus cyprini* and *Lamproglena pulchella* were determined on three different fish species (*Barbus lacerta, Capoeta trutta* and *Capoeta umbla*) caught in Uzunçayır Dam Lake. *Myxobolus cyprini* was detected on the pectoral fin of *C. trutta*, and *L. pulchella* was found in the gills of *B. lacerta*. No parasite was found in *C. umbla*. The parasites that were determined here were the first records for Uzunçayır Dam Lake.

Myxobolus cyprini Doflein, 1898

The cysts of *Myxobolus cyprini* was determined in the soft tissue of the pectoral fin of *Capoeta trutta* as nodules that are off-white colour, oval shape and located close to each other (Fig. 1). In the histopathological and microscopical examinations of the cysts, spores of *M. cyprini* with the typical character of the genus *Myxobolus* were observed. The mature spores of *M. cyprini* were ellipsoidal in frontal view (Fig. 2-3). The spore size of *M. cyprini* was measured as 6.17±0.40 µm in length, 4.74±0.69 µm in width and 1.95±0.26 µm in thickness. Two polar capsules were observed in the spore as pyriform and of equal size. The length and width of the polar capsules were measured 2.48±0.45 µm and 1.95±0.26 µm, respectively. Spores of *M. cyprini* were found infecting the skeletal muscle of *C. trutta* with a prevalence of 40 %.

The pathology results of the skeletal muscle of fish did not present macrophage accumulation signs and inflammation. The integrity of myofibrils within the infected fibers showed some degree of degeneration, with partial loss of myofibrillar details and striations. These pathological changes were determined in regions closed with cysts.

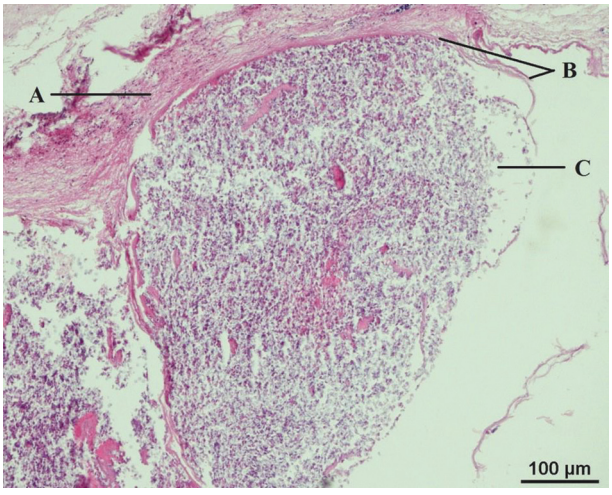

Figure 1. View of the cyst formed in the fın by *Myxobolus cyprini*. A: Connective tissue envelope, B: Ectoplasm, C: Endoplasm.

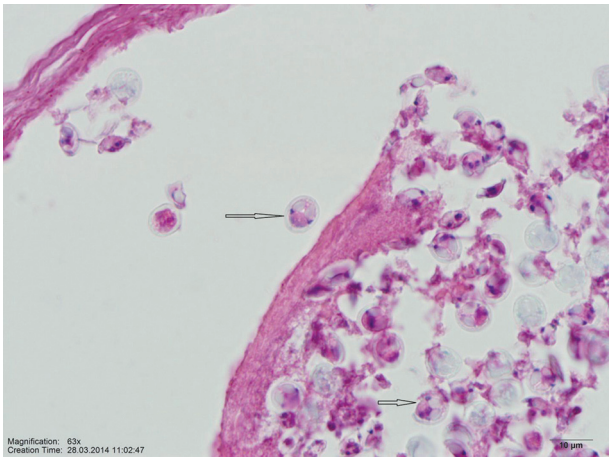

Figure 2. *The appearance of Myxobolus cyprini spores in the histological section of the first cyst in the fin.*

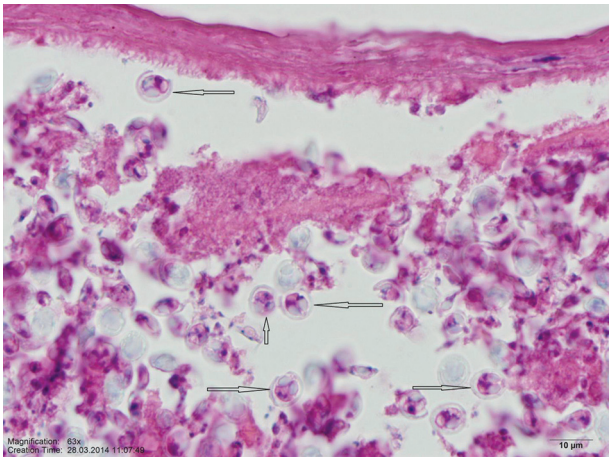

Figure 3. The a ppearance of *Myxobolus cyprini* spores in the histological section of the second cyst in the fin.

Lamproglena pulchella (Nordmann, 1832)

Lamproglena pulchella was found one pcs attached to the gill lamellae of *Barbus lacerta*. The body of *L. pulchella* was seen to have a cylindrical structure and consist of a conical abdomen, thorax and head (Fig. 4-6). Cephalothorax was separated from each other with a short neck. The first antennae were longer than the second antennae. The total length of the parasite is 3.01 mm, and its widest part was measured 0.51 mm. *L. pulchella* were found infecting the gills of *B. lacerta* with a prevalence of 33.33%.

In histopathological examinations of *L. pulchella* which is determined and revealed localised inflammation, characterised by swelling, haemorrhages and slight hyperplasia of connective tissue, epithelial desquamation, leukocyte infiltrations and deformations of the gills at regions where the parasite had attached.

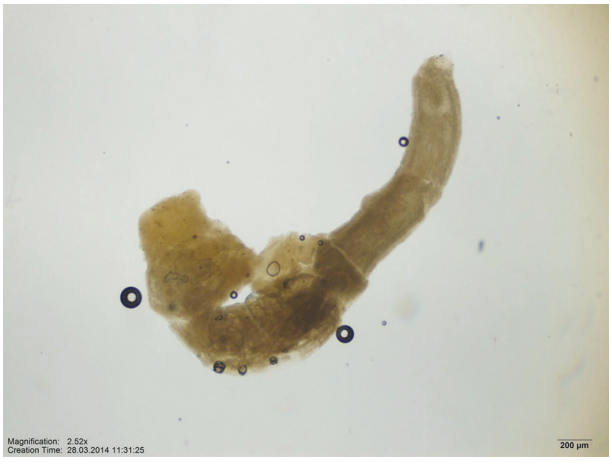

Figure 4. Total view of *Lamproglena pulchella*.

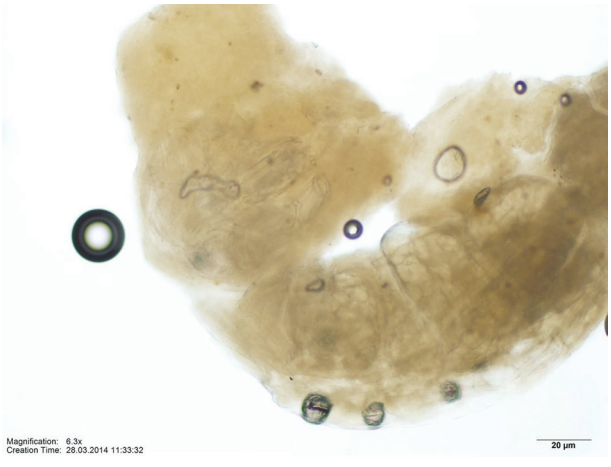

Figure 5. View of the cephalo-thorax of *Lamproglena pulchella*

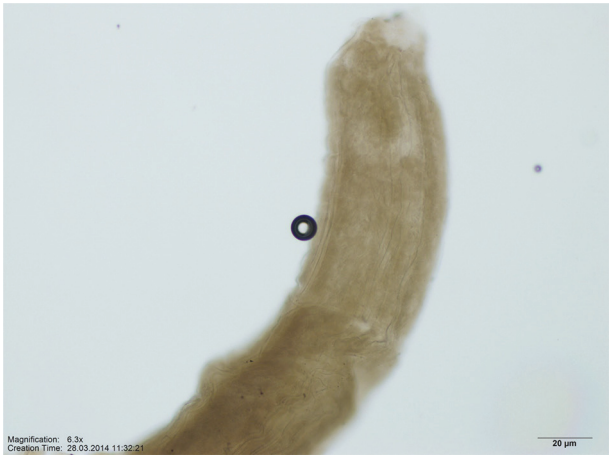

Figure 6. View of the abdomen of *Lamproglena pulchella*.

Discussion and Conclusion

Myxobolus cyprini, was found on the liver, spleen, kidney, gill, intestinal wall, skin and muscles of *Cyprinu scarpio* (Molnar and Kovacs-Gayer 1985). Although *M. cyprini* is known on the Cyprinidae family fish in Asia and Europe, it was first seen by Kent et al. (1996) in Western Hemisphere. The cysts of *M. cyprini* was collected from the fish species such as *Alburnus alburnus*, *Abramis brama*, *Blicca bjoerkna*, *Scardinius erythrophthalmus, Rhodeus sericeus amarus* Molnar et al. (2002) and *Leuciscus cephalus* Holzer and Schachner (2001) and their genetic identification was made. Dykova and Lom (1984) studied the variability and intra-muscular development of *M. cyprini* on the carps. In the present study, *M. cyprini* was determined as cysts in the soft tissue of the pectoral fin of *C. trutta*. These results showed that *M. cyprini* parasite cyst determined on *C. trutta* can also develop in soft tissues of fish fins. It was stated that the cysts of parasite species belonging to Myxosporea was in the form of white puffy dots on the gills and had the form of a small skin tumour and could be easily seen with the histological method . In the present study, the cysts of *M. cyprini* were found in a tumour form and could be easily seen spores with histological sections. Spores of *Myxobolus* sp. were found infecting the skeletal muscle of fish with a prevalence of 85.7% by Manrique et al. (2015). However, in our study, the prevalence of *M. cyprini* was found as 40%. it was less than half of prevalence data of Manrique et al. (2015)'s data. Also, pathological results of *Myxobolus cyprini* were similar to Manrique et al. (2015).

Although *L. pulchella* was determined on chub (*Squalius cephalus*) in many studies (Cakic et al. 1998; Galli et al. 2001a; Galli et al. 2001b; Jirsa et al. 2006) asp (*Aspius aspius*) and European catfish (*Silurus glanis*) (Molnár et al. 2018), it was found on *Barbus lacerta* in the present study. In all the studies carried out on *L. pulchella* (Jirsa et al. 2006; Jirsa et al. 2011; Galli et al. 2001a; Galli et al. 2001b; Cakic et al. 1998; Kus and Soylu 2013; Saglam 1998; Soylu 2012; Molnár et al. 2018) the parasite above was seen to prefer the gills of the fish and was not determined in another region. *L. pulchella* has been previously reported from Turkey in gills of *Capoeta trutta* and *Chondrostoma regium* in Keban Dam Lake (Saglam 1998); *Cyprinus carpio* and *Capoeta trutta* (Oktener et al. 2008) in Balıklıgöl; *Scardinius erythrophthalmus* in Sapanca Lake (Soylu 2012; Kus and Soylu 2013); *Squalius fellowesii* in the Doğanbaba Creek (Burdur) (Unal et al. 2017). As in the other studies, in this study, *L. pulchella* was found hanging on to the gill lamellae of the fish.

In this study, *L. pulchella* were determined as embedded in the gills of fish. This parasite on the gills of fish was previously recorded with a prevalence of 28.24% on *C. regium* and 28.50% on *C. trutta* (Saglam 1998), 20%-60% on *C. nasus* (Jirsa et al. 2006), 100% on both *C. carpio* and *C. trutta* (Oktener et al. 2008), 38.4%-88.8% on *S. erythrophthalmus* as seasonal (Soylu 2012), and 11.2% on *S. fellowesii* (Unal et al. 2017). It was less than half of the prevalence data of Oktener et al. (2008) and (Soylu 2012), while it was slightly higher data of Saglam (1998) and Unal et al. (2017). In addition, pathological results of *L. pulchella* were similar (Unal et al. 2017).

In conclusion, the determination of parasites of fish that live in their natural habitat shall contribute to knowing the parasitic risks in advance in the places, where cage fish farms shall be done, by taking necessary measures.

Ethic statement: Permission was obtained from the Elazığ Veterinary Control Institute Directorate Animal Experiments Local Ethics Committee (Approval number: 19.10.2012-10/1).

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