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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 11, Issue, 01, pp.10700-10707, January, 2020

RESEARCH ARTICLE

MOLECULAR IDENTIFICATION OF THE PARASITE LIGULA INTESTINALIS IDENTIFIED IN ALBURNUS ADANENSIS

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ARTICLE INFO

Article History:

Received 25th October, 2019
Received in revised form 29th November, 2019
Accepted 17th December, 2019
Published online 31st January, 2020

Key words:

Fish, *Alburnus adanensis,*
Parasite,
Ligula intestinalis,

ABSTRACT

This study covers the molecular identification of the *Ligula intestinalis* parasite identified in *Alburnus adanensis,* which was caught from Menzelet Dam Lake in Kahramanmaraş province. For this purpose, parasite samples were taken from the intestines of 60 *Alburnus adanensis* which were caught in January, February, March, April, May, June 2018. The parasites were stored in the sample bottles containing 70% alcohol. 27 *Ligula intestinalis* were identified by morphological and staining process. For isolating the DNA of *Ligula intestinalis*, a special tissue kit for parasites was used. Specific primers were used in the molecular identification of *Ligula intestinalis* by PCR technique. Therefore, all of these parasites were confirmed to be *Ligula intestinalis,* molecularly. As a result of this study, it was determined that the parasite *Ligula intestinalis* was found in *alburnus adanensis*, which was caught in Kahramanmaraş province. While the identification of the parasite *Ligula intestinalis* by morphological and staining methods took a long time, the identification of the parasite by PCR technique was accomplished in a short time. The success of this study will open the way for more original and comprehensive studies on molecular identification of parasitic agents in fish.

Citation: *Özcan M. and Kartal, B.* 2020. "Molecular identification of the parasite ligula intestinalis identified in alburnus adanensis", *Asian Journal of Science and Technology*, 11, (01), 10700-10707.

INTRODUCTION

In addition to its contribution to the national economy, the seafood sector plays an important role in terms of providing high levels of animal protein required for human nutrition. Societies that are conscious of a balanced and healthy diet make use of aquatic products at a high rate, especially fish, in meeting their animal protein needs (Arda *et al*., 2005). Fish is the healthiest source of protein for humans in many parts of the world, especially in developing countries. A large part of the world's protein needs are met by aquatic animal products. The current protein deficit and the deficit that is expected to grow gradually will be tried to be met from seafood again (Dick and Choudhury, 1995). Water products are produced by fishing or farming in marine and fresh waters. When we look at recent years, hunting and aquaculture in the world is constantly increasing. Technological developments play a major role in the decreasing trend in stocks due to the increase in fishing in marine and inland waters. A growing number of species live in lakes and seas with varying temperature and salinity rates in our country. Aquaculture is carried out in about 200 natural lakes, 300 dam lakes, 33 large rivers and 750 small ponds in our inland waters and provides a great contribution to the economy of the country. When we look at this potential in our country, it is of great importance to make the areas used in the fisheries sector active (ZMO, 2017). Although there is a significant amount of fishery activities in our country compared to other countries, there are very few fish. The Black Sea region is the leader in terms of fish production in our country. Besides this, fishing is also done in other regions. According to 2017 statistics, of the 354.318 tons of fish hunted, 322.173 tons are marine fish and 32.145 tons are freshwater fish (TÜİK, 2018). Fishery was examined in detail in the Keban Dam, Karakaya Dam, Atatürk Dam, Seyhan Dam, which are some of the important dam lakes of our country, and many other dams that are under construction, and information was provided about the amount of fish caught and which catching tools were used vermişlerdir (Duman and Çelik, 2001; Pala, 1996; Anonim, 1995; Avşar and Özyurt, 1999). In the studies carried out in our country's waters in recent years, parasitic diseases are frequently encountered in cyprinids found in natural waters and during aquaculture activities (Aksoy and Sarıeyyüpoğlu, 2000; Barata and Dörücü, 2014; Türk ve Dörücü, 2000; Dörücü and İspir, 2001; Dörücü and İspir, 2005; Dörücü *et al*., 2008; Gül *et al*., 2014; Sağlam and Sarıeyyüpoğlu, 2002; Buhurcu and Öztürk, 2007; Karabulut, 2009; Kılınçaslan, 2007; Kır and Tekin Özan, 2005; Küçükgül Güleç and Şahan, 2010; Özbek and Öztürk,

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2010; Özgül, 2008; Öztürk, 2012; Vılızzı *et al*., 2015). The species known as *Alburnus sellal* was recorded as *Alburnus adanensis*. *Alburnus adanensis*, which is found in the Seyhan and Ceyhan Rivers, is an endemic species (Fricke *et al*., 2007). The body structure of *Alburnus adanensis* is flattened from the sides. Its head is pointed and long when viewed from the top. The dorsal side of the mouth and between the ventral fin and the anal fin is a scaleless carina. It is characterized by a dark colored band extending from the back of the head to the base of the caudal fin (Erk'akan *et al*., 2009). Interest in aquaculture is increasing day by day throughout the world. However, fish diseases that affect reproduction, growth and nutrition cause great economic losses. For this reason, the recognition of diseases that pose a major problem for fish farming and the investigation of their treatments are of great importance (Dal, 2006). The issue of fish parasites is one of the most important issues affecting fish health in natural waters. Parasites that live in or on fish have mechanical, functional, toxic and exploitative effects, in addition to ingesting the fish's food. Parasitic agents sometimes affect only one fish, but often have negative consequences that lead to the loss of the existing stocks (Arda *et al*., 2005).

Aquatic environment is very important for the development and continuation of the life cycle of parasites. Parasitic infections are quite common in fish in the natural environment. However, pathological diseases caused by parasites are very rare in fish in aquaculture environment (Barber and Poulin, 2002). Since fish are at the top of the food chain in the water, they always face parasitic infection. When sufficient information is obtained about the parasites, the desired efficiency can be achieved by destroying the media that forms them and by tackling those that are much more dangerous (Tekin Özan *et al*., 2006). Parasitic diseases in fish are some of the health problems encountered in fish farming. In recent studies, it is stated that parasitic diseases are quite common in freshwater fish farming in our country. These diseases not only have harmful effects such as developmental retardation and reproductive problems in fish, they also lead to death in cases of dense parasite population (Stoskopf, 1984; Körting, 1984). The recognition of parasitic fish diseases and the investigation of their treatment are of great importance for the growing fishery sector today. In cases of high fish density and malnutrition, and when environmental conditions change, parasites are encountered intensively. Weakness and intense parasitic invasions developing with increasing stress factors can be fatal for fish (Arda *et al*., 2005; Çetin *et al*., 1983; Ekingen, 1983).

Sometimes even a parasite species can threaten existing stocks. Helminths are one of the parasitic groups that adversely affect aquatic ecosystems. Ligulosis caused by *Ligula intestinalis*, one of the pseudophyllidean cestodes, threatens the fishery works especially in the lakes and ponds of our country (Akkaya *et al*., 1998; Çolak, 1982; Güralp, 1974; Hoffman, 1967; Huet, 1979; Tolgay, 1973). *L. intestinalis*, classified in the Diphyllobothrium family, is a cestode that reaches 28 cm in adults and 40 cm in plerocercoids (Ciloğlu *et al*., 2013; Hajirostamloo, 2008). *Ligula intestinalis* is common in fresh water in the Northern Hemisphere, which includes Turkey. This parasite causes economic losses in the fish industry. Examples of skin, connective tissue and respiratory system of *L. intestinalis*.

This species of helminth is important for fish health and causes ligulosis (Ciloğlu *et al*., 2010; Hajirostamloo, 2008). *Ligula intestinalis* is common in species of the family Cyprinidae. This parasite is a type of pseudophyllidean cestode and has the general characteristics of cestodes. Scolex typically has dorsal and ventral bothriums. Sometimes bothriums are absent, and instead they have slits in the central part of the dorsal and ventral sides. The neck is quite short, almost absent. There are procercoid and plerocercoid stages in their development (Güralp, 1974; Hoffman, 1967; Tolgay, 1973). This is an endopacid species that causes productivity and death in the plerocercoid phase, especially in fish. Their larvae (plerocercoids) are found in the intestines of waterfowl in freshwater fish. The eggs are expelled through feces. Coracidia develop in water and are released. The first intermediate host is a freshwater fish, which thrives in Crustacea (Cyclops, water fleas as Diaptomus), and the second intermediate host is procercoids taken by the plerocercoid (Selver, 2008). The most important symptom of infection caused by *Ligula intestinalis* in fish is the swelling of the abdomen. The degree of swelling varies depending on the number and size of plerocercoids it carries. When the infection is severe, the pressure of this parasite is so high that the bellies of the fish burst and the plerocercoid larvae are released. The parasite restricts the function of the organs by pressurizing the fish's internal organs; with this effect, gonads can not function, and infertility and pernicious anemia can occur in fish (Akkaya *et al*., 1998; Çolak, 1982; Güralp, 1974; Hoffman, 1967; Huet, 1979; Sweeting, 1977; Timur and Timur, 2003).

In fish, the infection caused by the larva of *Ligula intestinalis* is called Ligulosis (Timur and Timur, 2003). In the fight against ligulosis, the treatment of fish bearing Ligler plerocercoids is not possible. It is not practical, and almost impossible to fight cyclops and diaptomus, the first intermediate host. In order to reduce the number of adult parasite-carrying and fish-eating waterfowls, measures should be taken at specific periods around lakes. Infected fish that act passively in water should be collected and destroyed as much as possible. During its larval stage, Ligula inhabits the abdominal cavity of various fish species, and rings are formed while they are here (Akkaya *et al*., 1998; Arda *et al*., 2005; Çolak, 1982; Güralp, 1974; Hoole, 1994; Huet, 1979; Tolgay, 1973). Molecular techniques are used to identify species that cannot be distinguished morphologically according to their genetic differences (Aksakal and Erdoğan, 2007). PCR is the process of amplification of DNA sequences in vitro under appropriate conditions. It is an in vitro method defined as enzymatically synthesizing copies of a particular piece of DNA guided by structures called primers. In PCR, millions or even billions of copies of the DNA molecule are produced in a short time. Thus, it is a technique that provides high sensitivity detection (Saiki *et al*., 1988; Mullis, 1990; Bej *et al*., 1991; Taylor, 1993; Marx, 1988; Coote, 1990; Quirke, 1992; Arda, 1995; Arı, 1999). Nuclear 28S rDNA and mitochondrial cytochrome c oxidase subunit I (COI) gene sequencing of *Ligula intestinalis* is used for molecular identification of this parasite (Li *et al*., 2000). In the event that both the existing fish species in aquatic ecosystems and the species that are grown intensively in net cages being exposed to this parasitic effect, molecular identification is important in order to prevent economic depreciation and to take the necessary measures.

Therefore, it was aimed to identify the parasite *Ligula intestinalis* identified in *Alburnus adanensis*, which was caught from Menzelet Dam Lake in Kahramanmaraş province.

MATERIAL AND METHOD

Material

Menzelet Dam is located on the Ceyhan River. This dam is located approximately 27 km (road distance) to the northwest of Kahramanmaraş city center. The GPS coordinates of the Menzelet Dam is 37° 40′ 31″ North and 36° 50′ 56″ East. The location of *Menzelet Dam* is shown on the map as 37.6754 latitude and 36.8489 longitude. The Menzelet Dam was constructed for energy production purposes, and it began to hold water in 1989. The minimum volume of the lake is 532.60 hm^3 and the maximum volume is 2.088 hm^3. The dam lake has a large area of 4200 hectares and its deepest place is 136 meters. The average depth is 50 m (Paksoy, 2002). The map of the *Menzelet Dam* where the *Alburnus adanensis* specimens used in this study were collected is shown in Figure 3.1. The study was carried out on *Alburnus adanensis*, which can be caught with gillnets from Menzelet Dam Lake in January, February, March, April, May, and June 2018 with the help of fishermen. A total of 60 *Alburnus adanensis* were used in the study. Chromosomal DNA was synthesized using DNA isolation kit (NucleoSpin Tissue), to be used to amplify the genes to be cloned. All primers used in the study were obtained from Integrated DNA Technologies (IDT). The primers (COIA2 CATATGTTTTGATTTTTTGG ve COIB2 AKAACATAATGAAAATGAGC) (Bouzid *et al*., 2008) were diluted to an average of 25-50 pmol for each PCR sample.

Method

60 *Alburnus adanensis*, which were caught from Menzelet Dam Lake, were transported to the Fish Diseases Laboratory of Fisheries Department of Faculty of Agriculture, Kahramanmaraş Sütçü İmam University, alive in plastic buckets and anesthetized with 2-phenoxyethanol. Species of fish were identified according to Geldiay and Balık (1996). Autopsies were performed according to the information given by Arda *et al*. (2005). In order to search for endoparasites, ventral incision was performed with the help of a small and fine tipped scissors starting from the anus in the abdominal part of the fish. The digestive tract was then taken to a black waxed petri plate, cut lengthwise from one end to the other by means of the same scissors and stretched on the waxed ground with needles. The digestive tract prepared in this way was examined under a binocular stereo microscope with the help of fine needles and brushes. After the parasites were counted and the organ from which they were obtained was recorded, they were removed with the help of brushes and fine needles and placed in petri plates containing 0.9% physiological salt solution. The parasites removed from the resting solution were fixed after their examination under light microscope. For later studies, the parasites were stored in Eppendorf tubes containing 70% ethyl alcohol. In order to identify species morphologically from Plerocercoid *Ligula intestinalis* samples, the structure and position of the internal organs should be known by taking the transverse section. Therefore, it is necessary to take transversal cross-section from the plerocercoids with a microtome. For microtome sectioning, plerocercoid fragments of 1 cm length were passed through series of 100% absolute alcohol, absolute alcohol-xylol (1:3, 1:1, 3:1) and 100% xylol, respectively. It was then blocked with paraffin and allowed to freeze for 1 day. A 7µ-thick transversal section was obtained from the specimen blocks with a sliding microtome. After staining the plerocercoid sections with hematoxylin eosin, permanent slides were made with them. The slides were dried at room temperature for 24 hours and examined under light microscope. Acanthocephala were fixed by taking them directly into AFA solution after careful cleaning with the brush. After waiting 3-7 days in this solution, they were taken to 70% ethyl alcohol and stored for a long time. The parasite samples were taken to Semichon's acetocarmine stain medium after fixation. As a result of the staining process, the anatomical structures of the parasites were examined in detail as a result of their body absorbing different concentrations of stain. Following staining, dehydration was carried out with the help of alcohol series (% 35, 50, 70, 85, 95, absolute alcohol) in order to remove the water in the parasite, and the transparentization process was started with the help of xylol and lactophenol for more clear examination of the internal tissue structures. For the sealing process of the material prepared according to these methods described, the slide was thoroughly cleaned and a drop of slide sealing (Canadian Balsam) was dropped into the center. The material in the transparentizing agent was taken with the help of a brush or a wire and placed inside the sealing medium, which was then covered with care to avoid air bubbles forming at one corner of the sealing medium. Pressure was applied to the slide with a clamp to fix the material flat. The slide was allowed to dry at room temperature. The parasite species and stock number were written at one corner of the dried slide, and the location where the host lived, host species, the organ where the parasite was found, and the date of examination were written at the other corner (Merdivenci, 1984; Williams and Jones, 1994).

The parasites were identified using the study of Bauer (1987) and Ekingen (1983). The parasite samples were taken into 70% alcohol and placed into Eppendorf tubes containing sterile distilled water. The alcohol on the parasite was removed by keeping it in sterile distilled water for 24 hours. Each parasite sample was cut with sterile scissors to be 0.2 g. These parasitic fragments were broken down with sterile crushing apparatus in Eppendorf and centrifuged for 10 minutes at 5000 rpm with 2 ml of sterile pure water added on them. After centrifugation, the water at the top of the Eppendorf tube was discarded. On top of the remaining pellet at the base of the Eppendorf tube, 20 µl of proteinase K and 180 µl of lysine buffer T1 were added, and mixed. These samples were incubated for 1 hour at 56 ^oC in shaking incubator. It was mixed in a vortex for 15 seconds, and 200 µl of lysine buffer B3 was added, and then It was mixed again in the vortex. 200 µl of ethyl alcohol BE was added and vortexed again. This mixture was transferred to the spin column tube contained in the collection tube and centrifuged at 10.000 rmp for 1 minute. The lower tube containing the liquid was emptied. The emptied tube was again placed under the spin column. 500 µl of wash buffer BW was added and centrifuged at 8.000 rpm for 1 minute. The collected liquid at the bottom of the spin column was removed. 500 µl of wash buffer B5 was added to the spin column and centrifuged at 12.000 rmp for 3 minutes. The collected liquid tube at the bottom of the spin column was discarded and a new Eppendorf tube was inserted. 200 ell of elution buffer was added to the spin column and incubated for

2 minutes at room temperature. It was then centrifuged for 1 minute at 8.000 rpm. It was poured into the spin column and stored in the Eppendorf tube at the bottom at -20 °C. In the PCR mixture prepared in a total volume of 50 µl, 25 µl of Master mix, 1 µl of Forward Primer, 1 µl of Reverse Primer, 1 µl of DNA and 22 µ dH_2O were included in a total volume of 50 µl. Two specific pairs of primers belonging to *Ligula intestinalis* were used. 50 pmol and 1 µl of template DNA were added from each pair of these primers: COIA2 5'CATATGTTTTGATTTTTTGG3' and COIB2 5'AKAA CATAA TGAAAATGAGC3'. In PCR amplification, following 10 minutes of pre-denaturation at 94 °C, a total of 35 PCR cycles, 30 seconds of denaturation at 94 °C, 45 seconds of hybridization at 56 °C, 1 minute of DNA synthesis at 72° C and 12 minutes of final extension at 72 °C were performed. From the DNA products amplified in PCR, 7 µl was taken and mixed with a 3 µl blue orange loading solution and placed in wells on the previously prepared 1.5% agarose gel. DNA marker was left in the last well. The gel was stained with ethidium bromide (10 mg/ml) for 30 minutes after undergoing electrophoresis for 1.5 hours at 80 V in 1.5% agarose gel using Tris-boric acid-EDTA (TBE) buffer. In the dark room, under the ultraviolet transilluminator, they were photographed using the polaroid camera system by looking at the DNA band sizes (bp-base pair) specific to *Ligula intestinalis*.

RESULTS AND DISCUSSION

Menzelet dam lake is located on the migration route of some birds due to its geographical location. Of the 60 parasitological fish samples, 27 were infected by *Ligula intestinalis* 3 by *Neoechinorhynchus rutile* (Table 1). A total of 60 fishes were examined in this study. 26 *Ligula intestinalis* were identified with morphological and staining methods. *Ligula intestinalis* was identified in 16 samples taken from Menzelet Dam Lake. DNA isolation was performed with these isolates. These DNAs were amplified by PCR. These products then underwent electrophoresis on a 1.5% agarose gel. The gel was stained with ethidium bromide and the results were evaluated with ultraviolet transilluminator. In all 120 samples, *Ligula intestinalis*'s COIA2- COIB2 primers and 480 bp long bands (Figure 2). Thus, 16 of the 60 isolates were proved to have a total of 27 *Ligula intestinalis*. In this study, firstly, *Ligula intestinalis* collected from *Alburnus adanensis* species caught from Menzelet Dam Lake in Kahramanmaraş province were identified by classical morphological and staining method. The suspected *Ligula intestinalis* samples were successfully confirmed by Polymerase Chain Reaction technique. 27 *Ligula intestinalis* parasites were found in the intestines of 60 *Alburnus adanensis* caught from Menzelet Dam Lake. *Ligula intestinalis* species were identified according to Hoffman (1967), Bykhovskaya-Pavlovskaya *et al*. (1964), Ekingen (1983) and Kennedy (1974).

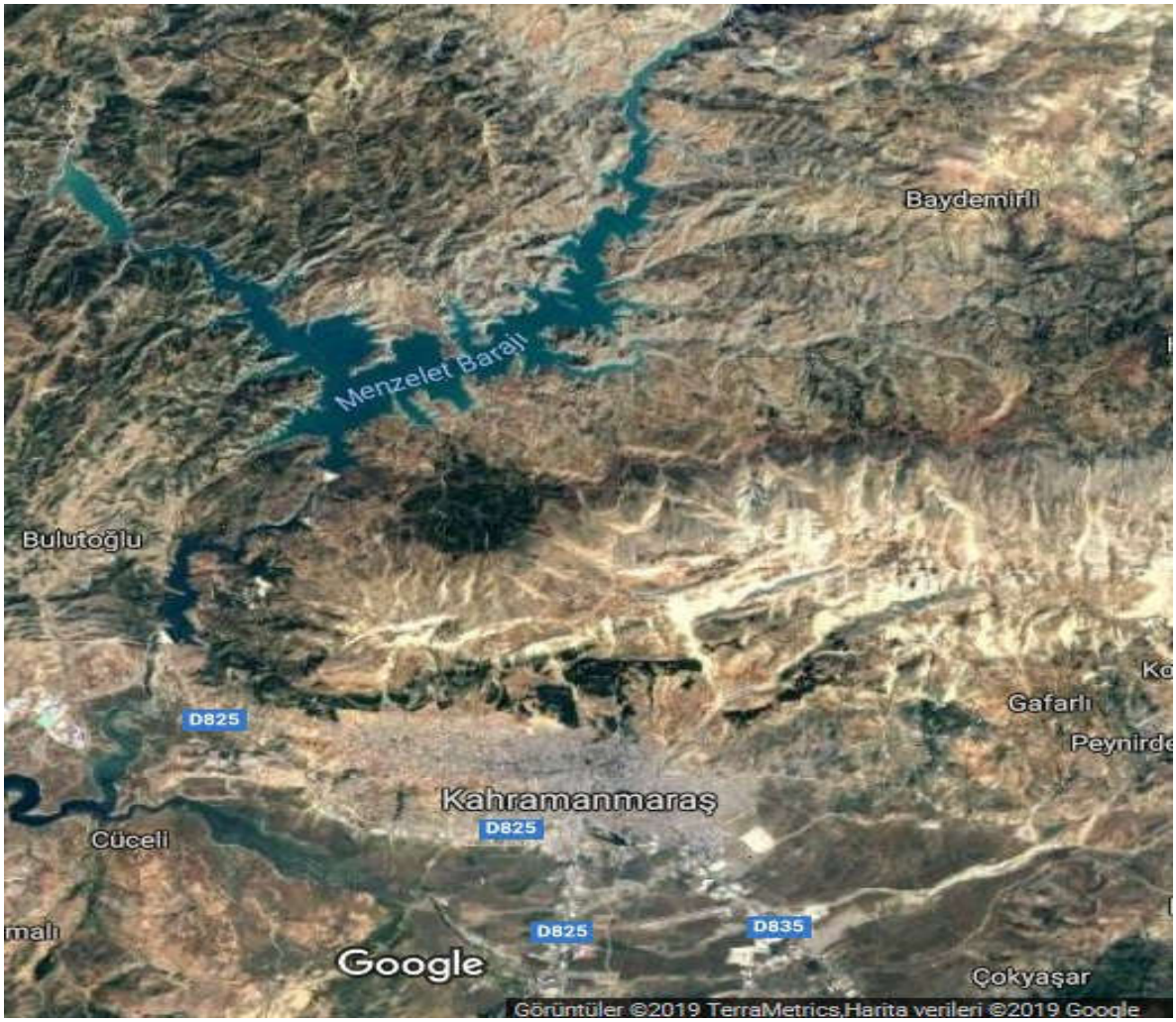

Figure 3.1. Menzelet Dam where *Alburnus adanensis* is hunted

Table 1. Samples of fish and parasites

NO	Net length	Prong length	Total length	Weight	sex	Parasitic number	Parasitic type	Location
1	12.3	11.3	10.5	25.2	D	0		
2	11.3	10.5	9.7	24.2	E	0		
3	10	9.1	8.3	23.6	E	0		
4	11.8	10.9	10.1	24.6	D	0		
5	9.3	8.4	7.7	22.8	E	2	*L.intestinalis*	Intestine
6	12.5	11.6	10.7	25.9	D	1	*N. rutili*	Intestine
7	14.2	13.2	12.2	27.8	D	0		
8	11.2	10.4	9.6	24	E	1	*L.intestinalis*	Intestine
9	8.8	8	7.2	21.3	D	1	*L.intestinalis*	Intestine
10	12.2	11.1	10.2	25.1	D	0		
11	15.5	14.6	13.7	28.5	E	1	*L.intestinalis*	Intestine
12	11.7	10.8	9.9	24.6	D	0		
13	16.8	15.9	14.8	29.7	E	0		
14	10.1	9.2	8.3	23.2	E	0		
15	17.4	16.5	15.6	31.5	D	0		
16	12.6	11.7	10.8	25.8	D	1	*L.intestinalis*	Intestine
17	18	17.1	16.2	32.9	E	1	*N. rutili*	Intestine
18	13.6	12.7	11.8	26.3	D	0		
19	16.5	15.6	14.7	30.2	D	0		
20	9.4	8.5	7.8	22,9	E	0		
21	12.7	11.8	10.9	26.1	D	0		
22	14.3	13.3	12.3	27.9	E	0		
23	11.5	10.7	9.9	24.3	E	0		
24	8.9	8.1	7.3	21.4	D	1	*L.intestinalis*	Intestine
25	12.4	11.3	10.4	25.3	E	1	*N. rutili*	Intestine
26	15.4	14.5	13.6	28.4	E	0		
27	11	10.1	9.2	23.9	D	4	*L.intestinalis*	Intestine
28	16.9	16	14.9	29.8	E	2	*L.intestinalis*	Intestine
29	10.2	9.3	8.4	23.3	D	0		
30	17.5	16.6	15.7	31.6	D	2	*L.intestinalis*	Intestine
31	16.7	15.8	14.7	29.6	E	5	*L.intestinalis*	Intestine
32	10.3	9.5	8.5	23.4	D	0		
33	17.6	16.7	15.8	31.7	E	0		
34	12	11.5	10.6	25.6	D	0		
35	18	17.1	16.2	32.9	D	0		
36	13.6	12.7	11.8	26.3	E	0		
37	16.5	15.6	14.7	30.2	D	0		
38	9.3	8.4	7.7	22.8	E	0		
39	12.7	11.8	10.9	26.2	E	0		
40	14.4	13.4	12.4	28	D	0		
41	11.6	10.8	10	24.4	E	0		
42	8.7	7.9	7.1	21.2	D	0		
43	12.5	11.4	10.5	25.4	D	0		
44	12.1	11.2	10.4	25.1	E	0		
45	11.4	10.6	9.8	24.3	D	0		
46	10.4	9.5	8.8	24	E	2	*L.intestinalis*	Intestine
47	11.9	11	10.2	24.7	E	1	*L.intestinalis*	Intestine
48	9.5	8.6	7.9	23	D	1	*L.intestinalis*	Intestine
49	12.8	11.9	11	26,2	E	1	*L.intestinalis*	Intestine
50	14.5	13.5	12.5	28.1	D	0		
56	16.6	15.7	14.6	29.5	D	0		
57	10.5	9.6	8.7	23.6	D	0		
58	17.7	16.8	15.9	31.8	E	2	*L.intestinalis*	Intestine
59	15.7	14.8	13.9	28.7	D	0		
60	10.8	9.9	9	23.7	E	0		

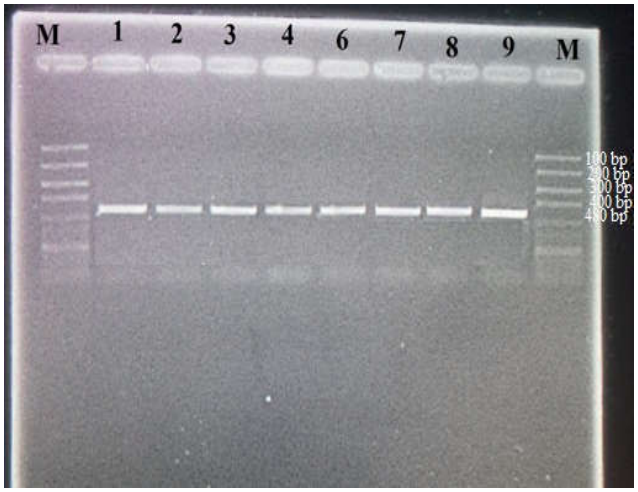

Figure 3. Analysis of PCR in DNA samples

They reported that they detected *Ligula intestinalis* parasite in their studies in freshwater fish (Dörücü and İspir 2005; Sağlam and Sarıeyyüpoğlu 2002; Karabulut 2009; Karabulut 2011; Barata 2012). In this study, the detection of *Ligula intestinalis* parasite was in agreement with the studies in terms of detection of the parasite in freshwater fish. It is stated that the PCR technique identifies a small number of microorganisms in pure or mixed cultures in a short period of time, therefore it is more advantageous than culture and serological tests (Lin and Tsen, 1996). The use of the specific primers in PCR amplification makes identification easy. In this study, the use of *Ligula intestinalis* species-specific COIA2 5' CATATGTTTTGATTTTTTGG3' and COIB2 5'AKAACATAATGAAAATGAGC3' primer pairs (Bouzid *et al*., 2008) prevented the false positive reaction of the parasite. The fact that this technique was carried out with negative control in all steps and that no contamination occurred as a result showed that PCR technique was applied successfully. Certain nucleotides of 393 bp for the COI gene of the 7 Ligula sample were sequenced directly from each sample. Interestingly, no nucleotide variation was detected in sequences of the COI gene among the 7 Ligula samples studied (Li *et al*., 2000). Islam (2019) identified a single pattern and a reliable band in all of the gene sequences of the 6 *L. intestinalis* isolates identified in his study. The bands were 480 bp in size. No nucleotide variation was detected in any of the COI gene products of the samples. Only the 225 C nucleotide of sample L2-2 was found to be sequenced as a T nucleotide in all other samples. In support of the data of the above researchers, in this study similar *Ligula intestinalis* specific primers were used. A single pattern and a reliable band were recorded in all of the gene sequences of the 27 *L. intestinalis* isolates. The bands were 480 bp in size.

The life cycles of fish, the spread of parasites and the economic losses caused by them were noted years ago. However, information about fish parasites in our country is still not sufficient (Güralp, 1981). Therefore, mass deaths are seen in acute parasitic diseases in our country. In addition, in chronic parasitic diseases, there are excessive weakening of fish, decline in growth and significant problems in marketing. The money spent for the treatment of parasitic diseases and the feed costs incurred as a result of not being able to benefit from the feed they receive make the situation even worse.

In accordance with their biological development, parasites need other living things in part or throughout their lives. They continue their parasitic life cycles on or within the fish, one of these living things called host. During this period of life, they constantly affect the metabolism and vital functions of the host. Parasites living in the digestive tract also impair the secretory function of the host. All these effects lead to diseases and occasionally kill the host (Williams and Jones, 1994). In order to prevent parasitic diseases causing economic losses in fisheries, this study was carried out considering that it is necessary to investigate *Ligula intestinalis* parasite which is common in fish species in our region.

Conclusion

The success of this study will open the way for more original and comprehensive researches on molecular identification of parasitic disease agents in all regions of our country in the future.

Acknowledgement

This study is summary of the second author's master thesis. This work was presented as a oral presentation at International Congress on Domestic Animal Breeding Genetics and Husbandry– 2019 (ICABGEH-19), September 11-13, 2019, Prag- Czech Republic.

Ethical Approval: All animal studies were approved by the Animal Ethics Committee of Kahramanmaraş Sütçü Imam University, Faculty of Agriculture (KSÜZİRHADYEK) and Research Institute (Protocol number: 2017/01).

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