DOI 10.2478/helm-2018-0012

HELMINTHOLOGIA, 55, 251 – 255, 2018

Research Note

Infection status with plerocercoid of ligulid tapeworm in cyprinid fish from three lakes in Republic of Korea

H. B. SONG[1], G. M. PARK[2]*

[1]Korea Fresh-Water Fish Ecology Institute, Chuncheon, Korea; [2]*Department of Environmental Medical Biology, and Institute for Clinical and Translational Research, Catholic Kwandong University College of Medicine, Gangneung, Korea, E-mail: *gmpark@cku.ac.kr*

Article info

Received January 22, 2018
Accepted March 1, 2018

Summary

We have investigated freshwater cyprinid fish for its current infection status with plerocercoid of ligulid tapeworm in the Republic of Korea. A total of 6,049 (517 Euiam Lake and 4,071 Hoengseong Lake in Gangweon-do and 1,461 Chungju Lake in Chungcheongbuk-do) fish were examined by dissecting the peritonial cavity between March 2015 and December 2016. Totally 45 (0.74 %) fish in of 5 (26.3 %) species (8 *Squalidus japonicus coreanus,* 6 *Squalidus gracilis majimae,* 7 *Opsariichthys uncirostris amurensis,* 15 *Zacco platypus* and 9 *Erythroculter erythropterus*) were infected with plerocercoids of ligulid tapeworm. The infection density with plerocercoids in *Erythroculter erythropterus* was 12 – 26 per fish infected in *Erythroculter erythropterus,* and 1 – 2 in other 4 fish species. The plerocercoid was ivory-white and 26.2 – 57.8 cm long. The prevalence value in this survey was 0.9 % (45/6,049). The genetic analysis in this study was conducted to identify plerocercoid species. Based on genetic analysis with data in GenBank, these plerocercoids were identified as the *L. intestinalis*.
Keywords: plerocercoid tapeworm; Chungju; Euiam and Hoengseong Lake

Introduction

The tapeworm *Ligula intestinalis* (Cestoda: Pseudophyllidea) is the most common species of the genus *Ligula* (Bloch, 1782). This organism, which has a three-host life cycle, infects a range of fresh water species in its plerocercoid stage, particularly members of the Cyprinidae, and as its second intermediate host and has a widespread distribution throughout the northern hemisphere (Bauer & Stolyarov, 1961; Dubinina, 1980; Brown *et al.*, 2002; Hajirostamloo, 2009). A fish becomes infected through the ingestion of eating infected copepods. Once inside the fish, the tapeworm develops in the fish's body cavity into a larval stage known as a plerocercoid in the fish's body cavity, which goes on and to infect fish-eating birds such as herons and cormorants (Dubinina, 1980; Loot *et al.*, 2001; Ergonul & Altindag, 2005). The parasites persist in the guts of birds for only a few days where they sexual maturity and to reproduce (Dubinina, 1980). As reported for different infected cyprinid species, regardless of sex, season and age, the gonads of infected fish remain immature and only early germ cell stages are present (Arme & Owen, 1968; Hoole, 1994; Olson *et al.*, 2002). To date, only two papers have investigated *Ligula intestinalis* in Korea (Ryu & Lee, 1992; Sohn *et al.*, 2016). However, a large-scale surveys about the infection status of *Ligula* plerocercoids in a variety of cyprinid fish species have not been conducted in Korea lakes. Many species of the genus *Ligula* are difficult to identify satisfactorily, at least if it is based on morphological characteristics alone. These organisms are flat, unsegmented and have a tapering anterior end with two bothridia pleurocercoids from different

* – corresponding author

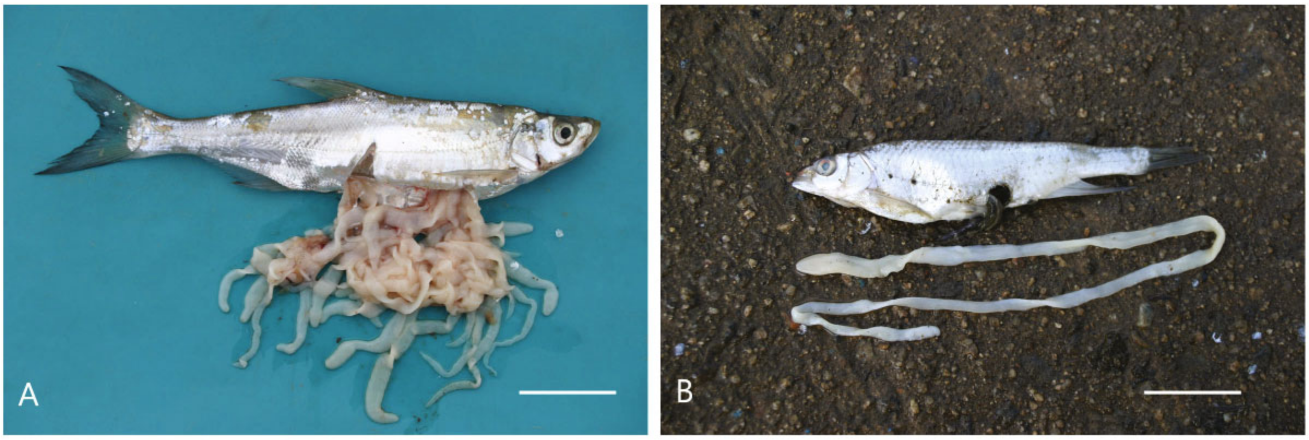

Fig. 1. An *Erythroculter erythropterus* with many plerocercoids of *Ligula intestinalis* from Chungju Lake(A) and an *Zacco platypus* with one plerocercoid from Hoengseong Lake (B). Scale bars are 5 cm.

host fish that vary in size from 10 – 100 cm in length, and 3 – 1.2 cm in width (Hajirostamloo, 2009). Recently, genetic analysis has been employed to distinguish *Ligula* species (Lee *et al.*, 2000; Li & Liao, 2003; Bouzid, 2008). Since *Ligula intestinalis* represents a complex of cryptic/sibling species, it is difficult to distinguish. Bouzid *et al*. (2008) and Štefka *et al*. (2009) have been reported that *Ligula intestinalis* seems to occur in Europe rather than in eastern Asia. The genetic analysis in this study was conducted to identify plerocercoid species. In the present study, we investigated the infection status of plerocercoid of the family Ligulidae from three lakes in Korea.

Materials and Methods

The infection status of freshwater cyprinid fish with *Ligula intestinalis* was examined in Chungju lake in Chungcheongbuk-do (do=Province), as well as Euiam and Hoengseong lakes in Gangwon-do, between March 2015 and December 2016. The fish were collected with the help of fisherman. We collected a total of 6,049 cyprinid fish belonging to 19 species from the three lakes once in every three months. The numbers and species of fish examined are shown in Table 1. Collected fish were transferred to the laboratory, classified to particular species and dissected to collect

Table 1. Infection status of cyprinid fish with plerocercoid of *L. intestinalis*, collected from Chungju, Euiam and Hoengseong lakes from Mar. 2015 to Dec. 2016.

Species	No. of fish collected from			Plerocercoid (mean no.)
	Euiam	Chungju	Hoengseong	
Carassius auratus	23	48	17	
C. cuvieri	2			
Cyprinus carpio	11	3		
Acheilognathus lanceolatus	25		8	
Acheilognathus yamatsutae	56	51		
Pseudorasbora parva	24	2	30	
Hemibarbus longirostris		3	4	
Pungtungia herzi	45	150	81	
S. nigripinnis.morii	28			
Squalidus japonicus coreanus	41	507(8*)		1 – 2
Squalidus gracilis majimae	22(6*)		27	1 – 2
Hemibarbus labeo	32	245		
H. longirostris	2			
M. yaluensis	3			
Pseudogobio esocinus	87	33	24	
Opsariichthys u. amurensis	21	69	1,816(7*)	1 – 2
Zacco platypus	95		2,064(15*)	1 – 2
Erythroculter erythropterus		311(9*)		12 – 26
Hemiculter leucisculus		39		
Total number of collected fish	517	1,461	4,071	

*: No. of infected fish with plerocercoid of *Ligula intestinalis*

Table 2. Number of infected fish with plerocercoid of *L. intestinalis*, collected from Chungju, Euiam and Hoengseong lakes from Mar. 2015 to Dec. 2016.

Date of fish collection	Number of infected fish			
	Euiam Lake	Hoengseong Lake	Chungju Lake	Total
20 Mar. 2015	1	1	1	3
21 June	1	0	1	2
14 Sep.	1	3	2	6
8 Dec.	0	8	3	11
19 Mar.	2	2	1	5
7 June	0	0	1	1
28 Sep.	0	2	3	5
3 Dec.	1	6	5	12
Total	6	22	17	45

the parasites. Plerocercoid present in the peritoneal cavity were counted, and identified using the characteristics suitable for species identification according to the criteria of Dubinina (Dubinina, 1980) and Bykhovskaya-Pavlovskaya et al. (Bykhovskaya-Pavlovskaya, 1962). Plerocercoid samples were preserved by either placing them in absolute ethanol or freezing them at -80 °C. Moreover, genetic analysis was conducted to identify plerocercoid species. Mitochondrial cytochrome *c* oxidase subunit I (*Cox 1*) and cytochrome *b* (*Cytb*) genes were used as targets (Bouzid, 2008). A total of 20 plerocercoid samples from five fish species were examined and compared with the GenBank-registered *Ligula* species.

Results

A total of 6,049 fish (517 from Euiam Lake, 1,461 from Chungju Lake and 4,071 from Hoengseong Lake) belonging to 19 species were examined by dissecting the peritoneal cavity in the present study (Table 1). Five of the 19 species of cyprinids fishes collected in three lakes were infected with plerocercoid of ligulid tapeworm. The infection status according to the fish species and surveyed localities is particularized in Table 1. Overall, totally 4,973 fish from five infected species in three lakes were investigated and the infection prevalence (0.9 %, 45/6.049) ranging from 0.2 % in January – July to 0.7 % in September – November (Table 2) was very low. The positive rates of fish with plerocercoids were as follows: 1.5 % (8) *Squalidus japonicus coreanus,* 12 % (6) *Squalidus gracilis majimae,* 0.4 % (7) *Opsariichthys uncirostric amurensis,* 0.7 % (15) *Zacco platypus* and 2.9 % (9) *Erythroculter erythropterus.* The plerocercoid was ivory-white and 26.2 – 57.8 cm long (Fig. 1). In *Erythroculter erythropterus*, the number of plerocercoid in the peritoneal cavity was 12 – 26, while the other four species had 1 – 2 plerocercoid in the peritoneal cavity. To obtain definitive information regarding the taxonomy of *Ligula* plerocercoid*,* the partial nucleotide sequence of mitochondrial *Cox 1* and *Cytb* genes were performed. The *Cox 1* (395 bp) and *Cytb* (405 bp) sequences obtained upon gene analysis were compared with GenBank data (Accession number; KY321843.1, KY321844.1, AF153910.1, EU241317.1, EU241316.1, EU241315.1, & EU241218.1, EU241217.1, EU241216.1, EU241215.1), and a 0.1 % genetic difference (2 – 4 bp) from *Ligula intestinalis* was observed. Based on comparison of the results of genetic analysis with data in GenBank, this plerocercoid was identified as *L. intestinalis*.

Discussion

It is well known that the tapeworm *Ligula intestinalis* infects many different species of freshwater fish, but primarily infects cyprinids (Sweeting, 1977; Barus & Prokes, 2002; Ergonul & Altindag, 2005). A fish becomes infected through the ingestion of infected copepods, after which the tapeworm develops into a larval stage called a plerocercoid in the fish's body cavity. The definitive hosts are ichthyophagous predatory birds such as herons and cormorants, in which *L. intestinalis* reaches sexual maturity. Parasite eggs are then released into the water with bird feces (Hoole, 1994). To date, a total of four species of freshwater fishes, *Zacco platypus, Carassius auratus, Hemiculter bleekeri* and *Chanodichthys erythropterus,* have been reported as the second intermediate hosts of *Ligula intestinalis* in Korea (Ryu & Lee, 1992; Sohn *et al*., 2016). *Ligula* pleurocercoids show very limited structural differentiation. Specifically, they are flat, unsegmented and have a tapering anterior end with two bothridia plerocercoids from different host fish that vary in size. Early accounts concerning the genus *Ligula* were reviewed by Cooper (1918), who recognized only the single species of *L. intestinalis*, and listed 63 species of the host fish from many different families (Arme & Owen, 1968; Hajirostamloo, 2009). Bykhovskaya-Pavlovskaya et al. suggested that at least two genera (*Digramma* and *Ligula*) and five species, including *L. intestinalis*, are involved and that the plerocercoid of each species is restricted to a relatively narrow range of host species. However, Luo et al (2003) compared *Digramma* and *Ligula* specimens based on ITS and the 5' end of 28S rDNA sequences and found that low level nucleotide variation between the two genera may imply that cestodes in the genus *Digramma* are paraphyletic to the *Ligula* genus, and that *Digramma* is a synonym of *Ligula*. To obtain definitive information regarding the taxonomy of *Ligula* plerocercoid obtained in this survey*,* we also analyzed the partial nucleotide sequence of mitochondrial *Cox 1* and *Cytb* genes. Based on comparison of the data obtained by genetic analysis to that in GenBank, these plerocercoids were identified as *L. intestinalis*.

The cyprinid fish that serve as the second intermediate hosts of this tapeworm have been reported in a variety of locations worldwide (*Alburnus filippi, Alburnoides bipunctatus, Capoeta capoeta, Cyprinus cpito*, *Gobio gobio, Salmo trutta*, *Scardinius erythrophthalmus, Leuciscus cephalus*, *Leuciscus leuciscus*, *Phoxinus phoxinus*, *Rutilus rutilus*, *Hemiculter bleekeri*, *Barbus* spp. and *Blicca bjoerkna*) (Arme & Owen, 1968; Charles & Orr, 1968; Hoole, 1994; Loot, 2001; Britton, 2009; Hajirostamloo, 2009; . Vanacker, 2012; Tizie *et al*., 2014; Sohn *et al*., 2016). The infected cyprinid fish confirmed in this study were *Squalidus gracilis majimae, Opsariichthys uncirostric amurensis, Zacco platypus, Squalidus japonicus coreanus* and *Erythroculter erythropterus*. The preponderance of infection in Korean cyprinids seems to be related to the widespread distribution and abundance of these fish. Although typically reported from cyprinid fish, *L. intestinalis* has been shown to utilize a broad range of hosts, including other fish families such as Catostomidae, Salmonidae or Galaxiidae (Dubinina, 1980; Groves & Shields, 2001; Barus & Prokes, 2002). Therefore, other freshwater families should be investigated in the future. *Ligula intestinalis* infections reportedly tend to occur between July and December (Loot *et al*., 2001). Infected fish in this study were found mainly among those caught in summer to winter. Thus, during autumn, *L. intestinalis* grows markedly in the host body cavity, and plerocercoids may reach a size between 10 and 30 cm long (Hoole, 1994). Since the worms in the bird host acquire maturity in 3 – 5 days in the bird host, infected piscivorous birds are rarely observed under natural conditions (Loot *et al.,* 2001). Thus, it is difficult to determine which bird species in the lakes play a key role in the transmission of *L. intestinalis* in the lakes. Accordingly, future studies investigating what kinds of bird are transmitting *L. intestinalis* in Korea are warranted.

References

Arme, C., Owen, R.W. (1968): Occurrence and pathology of *Ligula intestinalis* infections in British fishes. *J. Parasitol.*, 54(2): 272 – 280. DOI: 10.2307/3276934

Arme, C. (1968): Effects of the plerocercoid larva of pseudophyllidean cestode, *Ligula intestinalis*, on the pituitary gland and gonads of its host. *Biol. Bull*., 134(1): 15 – 25. DOI: 10.2307/1539963

Barus, V., Prokes, M. (2002): Length and weight of *Ligula intestinalis* plerocercoids (Cestoda) parasitizing adult cyprinid fishes (Cyprinidae): a comparative analysis. *Helminthologia*, 39: 29 – 34.

Bauer, O.N., Stolyarov, V.P. (1961): Formation of the parasite fauna and parasitic diseases of fishes in hydro-electric reservoirs. In: Dogiel, V.A., Petrushevski, G.K., Polyanski Yu.I. (Eds) *Parasitology of Fishes*. Oliver and Boyd: London, pp. 246 – 254

Bouzid, W., Štefka, J., Hypša, V., Lek, S., Scholz, T., Legal, L., Ben Hassine, O., Loot, G. (2008): Geography and host specificity: Two forces behind the genetic structure of the freshwater fish parasite *Ligula intestinalis* (Cestoda: Diphyllobothriidae). *Int. J. Parasitol*., 38(12): 1465 – 1479. DOI: 10.1016/j.ijpara.2008.03.008

Britton, J.R., Jackson, M.C., Harper, D.M. (2009): *Ligula intestinalis* (Cestoda: Diphyllobothriidae) in Kenya: a field investigation into host specificity and behavioral alterations. *Parasitology*, 136(11): 1367 – 1373. DOI: 10.1017/S003118200999059X

Brown, S.P., Loot, G., Teriokhin, A., Brunel, A., Brunel, C., Guégan, J.F. (2002): Host manipulation by *Ligula intestinalis*: a cause or consequence of parasite aggregation? *Int. J. Parasitol*., 32(7): 817 – 824. DOI: 10.1016/S0020-7519(02)00013-9

Bykhovskaya-Pavlovskaya, I.E., Gusev, V., Dubinina, M.N., Izyumova, N.A., Smirnova, T.S., Sokolovskaya, I.L., Shtein, G.A., Shul'man, S.S., Epshtein, V.M., Nagibina, L.F., Raikova, E.V., Strelkov, Y.A. (1962): *Key to the parasites of the freshwater fishes of the U.S.S.R*. Akademiya Nauk SSSR, Moscow, 776pp. (In Russian)

Charles, G.H., Orr, T.S.C. (1968): Comparative fine structure of outer tegument of *Ligula intestinalis* and *Schistocephalus solidus*. *Exp. Parasitol*., 22: 137 – 149. DOI: 10.1016/0014-4894(68)90087-8

Cooper, A.R. (1918): North American pseudophyllidean cestodes from fishes. III. *Biol. Monogr*., 4: 1 – 243. DOI: 10.5962/bhl.title.33563

Dubinina, M.N. (1980): *Tapeworms (Cestoda, Ligulidae) of the Fauna of the U.S.S.R.* American Publishing Company, Springfield, Va. New Delhi, 320pp.

Ergonul, M.B., Altindag, A. (2005): The occurrence and dynamics of *Ligula intestinalis* in its cyprinid fish host, tench, *Tinca tinca*, in Mogan Lake (Ankara, Turkey). *Vet. Med. Czech*. 50(12): 537 – 542

Groves, K.L., Shields, B.A. (2001): Observations on the plerocercoid stage of the tapeworm *Ligula* in three species of fish from the lower crooked river of central Oregon. *J. Aquat. Anim. Health*, 13(3): 285 – 289. DOI: 10.1577/1548-8667(2001)013<0285:OOTPSO>2.0.CO;2

Hajirostamloo, M. (2009): A report on occurrence and parasite-host of *Ligula intestinalis* in Sattarkhan Lake (East Azerbaijan-Iran). *Inter. Schol. Sci. Res. & Innovation*, 3(9): 458 – 461. urn:dai:10.1999/1307-6892/3861

Hoole, D. (1994): *T*apeworm infections in fish: past and future problems. In: Pike, A.W., Lewis, J.W. (Eds) *Parasitic diseases of fish.* Wales, UK: Samara Publishing Limitid, Tresaith, pp.119 – 140

Li, J., Liao, X., Yang, H. (2000): Molecular characterization of a parasitic tapeworm (*Ligula*) based on DNA sequences from formalin-fixed specimens. *Biochem. Genet*., 38(9 – 10): 309 – 322. DOI: 0006-2928/00/1000-0309

Li, J., Liao, X. (2003): The taxonomic status of *Digramma* (Pseudophyllidae: Ligulidae) inferred from DNA sequences*. J. Parasitol*., 89(4): 792 – 799. DOI: 10.1645/GE-3078

Loot, G., Brosse, S., Lek, S., Guégan, J.-F. (2001): Behaviour of roach (*Rutilus rutilus* L.) altered by *Ligula intestinalis* (Cestoda: Pseudophyllidea): a field demonstration. *Freshwater Biol*., 46(9): 1219 – 1227. DOI: 10.1046/j.1365-2427.2001.00733.x

Luo, H.Y., Nie, P., Yao, W.J., Wang, G.T., Gao, Q. (2003): Is the genus *Digramma* synonymous to the genus *Ligula* (Cestoda:

Pseudophyllidea)? Evidence from ITS and 5' end 28S rDNA sequences. *Parasitol. Res*., 89(5): 419 – 421. DOI:10.1007/s00436-002-0802-5
Olson, P.D., Littlewood, D.T., Griffiths, D., Kennedy, C.R., Arme, C. (2002): Evidence for the co-existence of separate strains or species of *Ligula* in Lough Neagh, Northern Ireland. *J. Helminthol*. 76(2): 171 – 174. DOI:10.1079/JOH2001101
Ryu, S.S., Lee, H.J. (1992): *Study on the Ligula intestinalis larvae parasitized in freshwater fish*. The 38th Korea Science Exihibition, Biological (Animal) Section, pp 1 – 14 (In Korean)
Sohn, W.M., Na, B.K., Jung, S.G., Kim, K.H. (2016): Mass death of predatory carp, *Chanodichthys erythropterus*, induced by plerocercoid larvae of *Ligula intestinalis* (Cestoda: Diphyllobothriidae). *Korean J. Parasitol.* 54(3): 363 – 368. DOI:10.3347/kjp.2016.54.3.363
Štefka, J, Hypša, V, Scholz, T. (2009): Interplay of host specificity and biogeography in population structure of a cosmopolitan endoparasite: microsatellite study of *Ligula intestinalis* (Cestoda). *Mol Ecol* 18(6): 1187 – 1206. DOI:10.1111/j.1365-294X.2008.04074.x
Sweeting, R.A. (1977): Studies on *Ligula intestinalis* some aspects of the pathology in the second intermediate host. *J. Fish. Biol*. 10(1): 43 – 50. DOI: 10.1111/j.1095-8649.1977.tb04040.x
Tizie, E., Baye, D., Mohamed, A. (2014): Prevalence of *Ligula intestinalis* larvae in Barbus fish genera at Lake Tana, Ethiopia. *World J. Fish Marine Sci.* 6(5): 408 – 416. DOI: 10.5829/idosi.wjfms.2014.06.05.85200
Vanacker, M., Masson, G., Beisel, J.N. (2012): Host switch and infestation by *Ligula intestinalis* L. in a silver bream (*Blicca bjoerkna* L.) population*. Parasitology,* 139(3): 406 – 417. DOI: 10.1017/S003118201100206X