





Survey of Zoonotic Trematode Metacercariae in Fish from Water systems of Geum-gang (River) in Republic of Korea

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Abstract: The infection status of zoonotic trematode metacercariae (ZTM) was surveyed in freshwater fishes from the water systems of Geum-gang (River) in the Republic of Korea (Korea). A total of 1,161 freshwater fishes from 6 local sites of Geum-gang were examined with the artificial digestion method for 4 years (2012-2015). *Clonorchis sinensis* metacercariae were detected in 122 (37.2%) out of 328 fishes in the positive fish species from 4 surveyed areas, and their mean intensity was 43 per fish infected. *Metagonimus* spp. metacercariae were found in 432 (51.7%) out of 835 fishes in the positive fish species from all 6 surveyed areas, and their mean intensity was 30 per fish infected. *Centrocestus armatus* metacercariae were detected in 285 (75.0%) out of 380 fishes in the positive fish species from 6 surveyed areas, and their mean intensity was 2,100 per fish infected. *Echinostoma* spp. metacercariae were found in 56 (19.7%) out of 284 fishes in the positive fish species from 5 surveyed areas, and their mean intensity was 10 per fish infected. *Clinostomum complanatum* metacercariae were detected in 98 (57.3%) out of 171 fishes in the positive fish species from only 2 surveyed areas, and their mean intensity was 11 per fish infected. Conclusively, the endemicity of ZTM is not so high in fishes from water systems of Geum-gang in Korea although it is more or less different by fish species, surveyed areas and ZTM species.

Key words: Zoonotic trematode metacercaria, *Clonorchis sinensis*, *Metagonimus* spp., *Centrocestus armatus*, *Echinostoma* spp., *Clinostomum complanatum*, Geum-gang

INTRODUCTION

Nowadays, fishborne zoonotic trematodes (FZT) including *Clonorchis sinensis* are the most important helminth group in the Republic of Korea (Korea) [1]. A team of Korean CDCP (Centers for Disease Control and Prevention) has performed the control project for zoonotic trematode infections in some endemic areas, especially, riverside areas of 7 major rivers, i.e., Han-gang (gang means river), Geum-gang, Mangyeong-gang, Yeongsan-gang, Tamjin-gang, Seomjin-gang and Nakdong-gang, in Korea [2-5]. The co-working groups of CDCP have also examined freshwater fishes, the infection sources of FZT, from the water systems of major rivers in Korea to reveal the

infection status of zoonotic trematode metacercariae (ZTM) [6-13].

Many Korean workers have performed epidemiological surveys on the infection status of ZTM in freshwater fishes, the human infection sources, from a variety of ecological environments, i.e., river, stream, lake, pond and swamp, to estimate the endemicities of ZTM [6-14]. Especially, Cho et al. [6] investigated the infection status of *C. sinensis* metacercariae (CsMc) in freshwater fish from 3 wide regions, which were tentatively divided by the latitudinal levels of the Korean peninsula. Cho et al. [7] also surveyed on the infection status of ZTM in freshwater fish from Gangwon-do (do=Province), Korea. Sohn et al. [8] investigated the infection status of digenetic trematode metacercariae (DTM) including *C. sinensis* in freshwater fish from the water systems of Hantan-gang and Imjin-gang in northern regions of Korea. Sohn et al. [9] and Yoon et al. [10] reported the infection status of CsMc in freshwater fishes from the water systems of Seomjin-gang and Tamjin-gang. Sohn et al. [11,12] also reported the infection status of CsMc in fresh-

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water fish from 2 highly endemic sites, Wi-cheon (cheon means stream) and Yang-cheon (branch streams of Nakdong-gang), in Gunwi-gun (gun=county), Gyeongsangbuk-do and Sancheong-gun, Gyeongsangnam-do, Korea. Recently, Sohn and Na [14] described the infection status of DTM in freshwater fishes from 2 visiting sites, Junam-jeosuji (jeosuji means reservoir) and Woopo-neup (neup means swamp), of migratory birds in Gyeongsangnam-do, Korea. Sohn et al. [13] also reported the infection status with DTM in fishes from coastal lakes in Gangwon-do, Korea.

Geum-gang is one of the major rivers in Korea, which originates in Jangsu-gun, Jeollabuk-do. It flows northward through Jeollabuk-do and Chungcheongbuk-do and then changes direction in the vicinity of Daejeon Metropolitan City, and flows southwest through Chungcheongnam-do and reaches the Yellow Sea near Gunsan-si, (si=city) Jeollabuk-do. This river has many tributary streams including Yugu-cheon and Nonsan-cheon [15]. On the other hand, several epidemiological studies have been performed to investigate the infection status of zoonotic trematodes, i.e., *C. sinensis* and *Metagonimus* spp., in the riverside areas of Geum-gang [16-21]. However, studies on the infections of ZTM in fish hosts were not enough. Kim [16] and Kim et al. [17] studied on the epidemiological and biological characteristics of *Metagonimus* sp. fluke, which was prevalent in the adjacent areas of Geum-gang at 1980's. Recently, Choe et al. [21] surveyed the infection status of DTM in 2 exotic fish species, *Micropterus salmoides* and *Lepomis macrochirus*, and some species of freshwater fish from 2 sites of Geum-gang in Chungcheongbuk-do, Korea. The large-scale survey on the infection status with ZTM in a variety of fish species has not been conducted yet in the areas of Geum-gang. Therefore, in the present study, we investigated the infection status of ZTM in freshwater fishes from 6 sites of Geum-gang in Jeollabuk-do and Chungcheongnam-do for 4 years (2012-2015).

MATERIALS AND METHODS

Fish collection sites

We collected total 1,161 freshwater fishes in 6 local sites of Geum-gang, i.e., Juja-cheon (Latitude: 35.980225; Longitude: 127.393880) in Jinahn-gun, Geum-gang (35.975291; 127.556624) in Muju-gun, Jeollabuk-do, Geum-gang (36.114265; 127.587748) in Geumsan-gun, Yugu-cheon (36.537272; 126.948474) in Gongju-si, Ji-cheon (36.389576; 126.851738) in Cheongyang-gun, Nonsan-cheon (36.199058; 127.067899) in Nonsan-si,

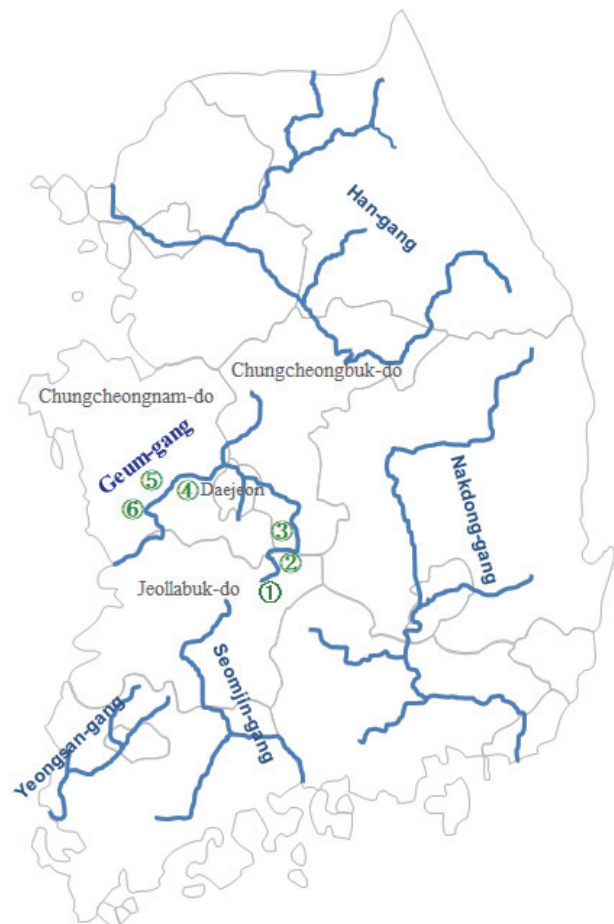


Fig. 1. The surveyed areas in the water systems of Geum-gang (River) in Jeollabuk-do and Chungcheongnam-do, Korea: ① Juja-cheon in Jinahn-gun, ② Geum-gang in Muju-gun, Jeollabuk-do, ③ Geum-gang in Geumsan-gun, ④ Yugu-cheon in Gongju-si, ⑤ Ji-cheon in Cheongyang-gun, ⑥ Nonsan-cheon in Nonsan-si, Chungcheongnam-do, Korea.

Chungcheongnam-do, for 4 years (2012-2015) (Fig. 1).

Fishes examined

Total 208 freshwater fish (18 species) from Juja-cheon in Jinahn-gun, Jeollabuk-do were examined in 2012. Species of fish examined (No. of fish) were *Zacco platypus* (40), *Zacco temminckii* (40), *Pungtungia herzi* (25), *Hemibarbus longirostris* (22), *Odontobutis platycephala* (22), *Squalidus gracilis majimae* (16), *Pseudogobio esocinus* (10), *Iksookimia longicarpus* (8), *Acheilognathus koreensis* (7), *Microphysogobio koeensis* (5), *Iksookimia hugowolfeldi* (3), *Liobagrus obesus* (2), *Silurus asotus* (2), *Pseudobagrus koreanus* (2), *Misgurnus anguillicaudatus* (1), *Pseudopungtungia nigra* (1), *Rhynchocypris oxycephalus* (1), *Carassius auratus* (1). In Geum-gang in Muju-gun, Jeollabuk-do, a total of 123

fishes (14 spp.) were examined in 2012. Species of fish examined (No. of fish) were *P. herzi* (35), *P. koreanus* (25), *Coreoperca herzi* (20), *Z. temminckii* (11), *P. esocinus* (10), *Pseudobagrus brevicorpus* (10), *H. longirostris* (4), *Sarcocheilichthys variegatus wakiyae* (2), *Acheilognathus lanceolatus* (1), *P. nigra* (1), *O. platycephala* (1), *C. auratus* (1), *Pseudobagrus fulvidraco* (1), *Siniperca scherzeri* (1).

Total 386 freshwater fish (22 species) from Geum-gang in Geumsan-gun were examined for 3 years (2013-2015). Species of fish examined (No. of fish) were *P. esocinus* (76), *H. longirostris* (67), *Z. platypus* (38), *C. herzi* (38), *Z. temminckii* (33), *O. platycephala* (23), *C. auratus* (20), *P. herzi* (15), *Acheilognathus yamatsutae* (11), *Liobagrus mediadiposalis* (10), *Acheilognathus majusculus* (10), *Squalidus japonicus coreanus* (10), *Sarcocheilichthys nigripinnis morii* (8), *S. scherzeri* (7), *Coreoleuciscus splendidus* (5), *S. variegatus wakiyae* (4), *Opsariichthys uncirostris amurensis* (4), *Hemibarbus labeo* (3), *Micropterus salmoides* (1), *S. gracilis majimae* (1), *Acanthorhodeus gracilis* (1), *Abbottina springeri* (1). In the Yugu-cheon in Gongju-si, a total of 311 fishes (14 spp.) were examined in 2013 and 2015. Species of fish examined (No. of fish) were *A. lanceolatus* (72), *Z. platypus* (59), *H. longirostris* (32), *P. esocinus* (28), *P. herzi* (22), *A. yamatsutae* (20), *O. uncirostris amurensis* (18), *C. auratus* (17), *Acheilognathus rhombeus* (15), *O. platycephala* (10), *A. springeri* (7), *S. variegatus wakiyae* (5), *H. labeo* (5), *S. gracilis majimae* (1).

Total 89 freshwater fish (13 spp.) from Ji-cheon in Cheongyang-gun were examined in 2014. Species of fish examined (No. of fish) were *Z. temminckii* (29), *H. longirostris* (13), *P. esocinus* (11), *S. scherzeri* (11), *A. koreensis* (10), *Z. platypus* (4), *P. herzi* (3), *C. auratus* (3), *S. nigripinnis morii* (1), *O. platycephala* (1), *Iksookimia koreensis* (1), *P. koreanus* (1), *P. fulvidraco* (1). In the Nonsan-cheon in Nonsan-si, a total of 44 fishes (11 spp.) were examined in 2013. Species of fish examined (No. of fish) were *Lepomis macrochirus* (10), *C. auratus* (10), *O. uncirostris amurensis* (7), *Hemiculter eigenmanni* (6), *S. scherzeri* (3), *Pseudorasbora parva* (2), *M. salmoides* (2), *P. herzi* (1), *Hemiculter leucisculus* (1), *S. variegatus wakiyae* (1), *P. esocinus* (1).

Method of fish examination

All collected fishes with ice were transferred to the laboratory of Department of Parasitology and Tropical Medicine, Gyeongsang National University College of Medicine, Jinju, Korea. Their length and weight were individually measured and identified the fish species [22]. Individual fish was finely ground in a mortar with pestle, the ground fish meat was

mixed with artificial gastric juice, and the mixture was incubated at 36°C for about 2 hr. The digested material was filtered with 1 × 1 mm² of mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined under a stereomicroscope. The metacercariae of each species (only ZTM) were separately collected viewing from the general feature, and were counted to get hold of the prevalence (%) and intensity of infection (No. of ZTM per fish infected) by fish species [23,24].

RESULTS

The metacercariae of *C. sinensis* were detected in 122 (37.2%) out of 328 fishes in the positive fish species from 4 surveyed areas, i.e., Geum-gang in Muju-gun, Jeollabuk-do, and Geum-gang in Geumsan-gun, Ji-chen in Cheongyang-gun and Nonsan-cheon in Nonsan-si, Chungcheongnam-do. Their mean intensity of infection was 43 per fish infected. Especially, in striped shiner, *P. herzi*, from Geum-gang in Muju-gun, Jeollabuk-do and in Geumsan-gun, Chungcheongnam-do, the prevalences were 97.1% and 100%, and the mean intensity of infection was 95 and 98 per fish infected. The infection status by the fish species and surveyed areas was shown in Table 1 in detail.

The metacercariae of *Metagonimus* spp. were found in 432 (51.7%) out of 835 fishes in the positive fish species from 6 surveyed areas, and their mean intensity of infection was 30 per fish infected. Especially, in goby minnow, *Pseudogobio esocinus*, from Geum-gang in Geumsan-gun, Chungcheongnam-do, the prevalence was 98.7% and the mean intensity of infection was 86 per fish infected. The infection status by the fish species and surveyed areas was designated in Table 2 in detail.

The metacercariae of *Centrocestus armatus* were detected in 285 (75.0%) out of 380 fishes in the positive fish species from 6 surveyed areas, and their mean intensity of infection was 2,100 per fish infected. Especially, in pale chub, *Zacco platypus*, from Yugu-cheon in Gongju-si, and Geum-gang in Geumsan-gun, Chungcheongnam-do, the prevalences were 100% in each and the mean intensity of infection were more than 7,000 and 3,300 per fish infected. The infection status by the fish species and surveyed areas was revealed in Table 3 in detail.

The metacercariae of *Echinostoma* spp. were detected in 56 (19.7%) out of 284 fishes in the positive fish species from 5 surveyed areas, and their mean intensity of infection was 10 per fish infected. Especially, in Korean dark sleeper, *O. platy-*

Table 1. Infection status of *Clonorchis sinensis* metacercariae (CsMc) in freshwater fish from Geum-gang in Jeollabuk-do and Chungcheongnam-do, Korea

Locality and fish sp.	No. of fish examined	No. (%) of fish infected	No. of CsMc detected	
			Range	Average
Geum-gang in Muju-gun, Jeollabuk-do				
<i>Pungtungia herzi</i>	35	34 (97.1)	1-141	95.2
<i>Sarcocheilichthys variegatus</i>	2	2 (100)	6-11	8.5
<i>Siniperca scherzeri</i>	1	1 (100)	-	2.0
Subtotal	38	37 (97.4)	1-141	88.0
Geum-gang in Geumsan-gun				
<i>Pseudogobio esocinus</i>	76	4 (5.3)	1-2	1.3
<i>Hemibarbus longirostris</i>	67	19 (28.4)	1-2	1.2
<i>Zacco platypus</i>	38	2 (5.3)	-	1.0
<i>Pungtungia herzi</i>	15	15 (100)	3-924	98.1
<i>Acheilognathus yamatsutae</i>	11	2 (18.2)	-	1.0
<i>Squalidus japonicus coreanus</i>	10	8 (80.0)	1-15	4.6
<i>Sarcocheilichthys nigripinnis</i>	8	7 (87.5)	1-69	14.4
<i>Coreoleuciscus splendidus</i>	5	2 (40.0)	1-6	3.5
<i>Sarcocheilichthys variegatus</i>	4	4 (100)	2-22	13.5
<i>Squalidus gracilis majimae</i>	1	1 (100)	-	28.0
Subtotal	235	64 (27.2)	1-924	27.0
Ji-cheon in Cheongyang-gun				
<i>Hemibarbus longirostris</i>	13	7 (53.9)	1-3	1.9
<i>Pseudogobio esocinus</i>	11	1 (9.1)	-	3.0
<i>Siniperca scherzeri</i>	11	2 (18.2)	1-2	1.5
<i>Pungtungia herzi</i>	3	3 (100)	8-81	50.7
<i>Sarcocheilichthys nigripinnis</i>	1	1 (100)	-	63.0
Subtotal	39	14 (35.9)	1-81	16.7
Nonsan-cheon in Nonsan-si				
<i>Opsariichthys uncirostris</i>	7	1 (14.3)	-	1.0
<i>Hemiculter eigenmanni</i>	6	4 (66.7)	1-3	2.0
<i>Pseudorasbora parva</i>	2	1 (50.0)	-	2.0
<i>Sarcocheilichthys variegatus</i>	1	1 (100)	-	1.0
Subtotal	16	7 (43.8)	1-3	1.7
Total	328	122 (37.2)	1-924	42.9

cephala, from Yugu-cheon, the prevalence was 100% and the mean intensity of infection was 48 per fish infected. The infection status by the fish species and surveyed areas was shown in Table 4 in detail.

The metacercariae of *Clinostomum complanatum* were detected in 98 (57.3%) out of 171 fishes in the positive fish species from only 2 surveyed areas, i.e., Geum-gang in Geumsan-gun and Yugu-cheon in Gongju-si, Chungcheongnam-do. Their mean intensity of infection was 11 per fish infected. Especially, in Korean striped bitterling, *Acheilognathus yamatsutae*, from Yugu-cheon, the prevalence was 95.0% and the mean intensity of infection was 18 per fish infected. The infection status by the fish species and surveyed areas was designated in Table 5 in detail.

DISCUSSION

In the present study, more than 5 species of ZTM, i.e., *C. sinensis*, *Metagonimus* spp., *C. armatus*, *Echinostoma* spp. and *C. complanatum*, were detected in fishes from the water systems of Geum-gang, but their endemicities were not so high. The metacercariae of *C. sinensis* were found in fishes from 4 out of 6 survey regions, and their overall prevalence and intensity of infection were 37.2% and 43 per fish infected in positive fish species. *Metagonimus* spp. metacercariae were detected in 51.7% fishes in positive fish species from 6 all survey regions, but their mean intensity was about 30 per fish infected. The metacercariae of *C. armatus* were found in fishes from all 6 survey regions, and they were heavily infected in pale chubs, *Z. platypus*, from Yugu-cheon in Gongju-si and Geum-gang in

Table 2. Infection status of *Metagonimus* spp. metacercariae (MsMc) in freshwater fish from Geum-gang in Jeollabuk-do and Chungcheongnam-do, Korea

Locality and fish sp.	No. of fish examined	No. (%) of fish infected	No. of MsMc detected	
			Range	Average
Juja-cheon in Jinahn-gun, Jeollabuk-do				
<i>Zacco platypus</i>	40	22 (55.0)	1-25	6.0
<i>Zacco temminckii</i>	40	33 (82.5)	1-16	3.9
<i>Pungtungia herzi</i>	25	4 (16.0)	1-2	1.3
<i>Hemibarbus longirostris</i>	22	4 (18.2)	1-2	1.3
<i>Squalidus gracilis majimae</i>	16	3 (18.8)	1-8	3.7
<i>Pseudogobio esocinus</i>	10	1 (10.0)	-	2.0
<i>Microphysogobio koeensis</i>	5	1 (20.0)	-	1.0
Subtotal	158	68 (43.0)	1-25	4.2
Geum-gang in Muju-gun, Jeollabuk-do				
<i>Pungtungia herzi</i>	35	2 (5.7)	1-2	1.5
<i>Zacco temminckii</i>	11	3 (27.3)	-	1.0
<i>Pseudogobio esocinus</i>	10	8 (80.0)	1-10	3.5
<i>Hemibarbus longirostris</i>	4	2 (50.0)	-	1.0
Subtotal	60	15 (25.0)	1-10	2.4
Geum-gang in Geumsan-gun				
<i>Pseudogobio esocinus</i>	76	75 (98.7)	1-457	85.7
<i>Hemibarbus longirostris</i>	67	66 (98.5)	1-82	18.4
<i>Zacco platypus</i>	38	37 (97.4)	7-275	74.3
<i>Zacco temminckii</i>	33	25 (75.8)	1-53	10.0
<i>Pungtungia herzi</i>	15	8 (53.3)	1-3	1.8
<i>Acheilognathus yamatsutae</i>	11	7 (63.6)	1-11	4.7
<i>Acheilognathus majusculus</i>	10	9 (90.0)	3-23	7.1
<i>Squalidus japonicus coreanus</i>	10	4 (40.0)	1-3	2.3
<i>Sarcocheilichthys nigripinnis</i>	8	3 (37.5)	1-3	2.3
<i>Opsariichthys uncirostris</i>	4	4 (100)	4-127	52.0
<i>Sarcocheilichthys variegatus</i>	4	2 (50.0)	1-2	1.5
<i>Acanthorhodeus gracilis</i>	1	1 (100)	-	4.0
Subtotal	277	241 (87.0)	1-457	45.6
Yugu-cheon in Gongju-si				
<i>Acheilognathus lanceolatus</i>	72	1 (1.4)	-	1.0
<i>Zacco platypus</i>	59	37 (62.7)	1-6	2.9
<i>Hemibarbus longirostris</i>	32	9 (28.1)	1-14	3.3
<i>Pungtungia herzi</i>	22	1 (4.6)	-	1.0
<i>Acheilognathus yamatsutae</i>	20	1 (5.0)	-	1.0
<i>Opsariichthys uncirostris</i>	18	1 (5.6)	-	1.0
<i>Carassius auratus</i>	17	1 (5.9)	-	9.0
<i>Acheilognathus rhombeus</i>	15	1 (6.7)	-	1.0
<i>Sarcocheilichthys variegatus</i>	5	4 (80.0)	1-6	2.5
Subtotal	260	56 (21.5)	1-14	2.9
Ji-cheon in Cheongyang-gun				
<i>Zacco temminckii</i>	29	16 (55.2)	1-25	6.9
<i>Hemibarbus longirostris</i>	13	10 (76.9)	9-332	4.1
<i>Pseudogobio esocinus</i>	11	10 (90.9)	3-150	36.3
<i>Zacco platypus</i>	4	4 (100)	1-60	16.8
<i>Pungtungia herzi</i>	3	2 (66.7)	2-4	3.0
<i>Carassius auratus</i>	3	2 (66.7)	5-10	7.5
<i>Pseudobagrus koreanus</i>	1	1 (100)	-	1.0
<i>Sarcocheilichthys nigripinnis</i>	1	1 (100)	-	2.0
Subtotal	65	46 (69.2)	1-332	32.7

(Continued to the next page)

Table 2. Continued

Locality and fish sp.	No. of fish examined	No. (%) of fish infected	No. of MsMc detected	
			Range	Average
Nonsan-cheon in Nonsan-si				
<i>Opsariichthys uncirostris</i>	7	3 (42.9)	2-13	6.3
<i>Hemiculter eigenmanni</i>	6	1 (16.7)	-	1.0
<i>Pseudorasbora parva</i>	2	2 (100)	1-4	2.5
Subtotal	15	6 (40.0)	1-13	4.2
Total	835	432 (51.7)	1-457	30.1

Table 3. Infection status of *Centrocestus armatus* metacercariae (CaMc) in freshwater fish from Geum-gang in Jeollabuk-do and Chungcheongnam-do, Korea

Locality and fish sp.	No. of fish examined	No. (%) of fish infected	No. of CaMc detected	
			Range	Average
Juja-cheon in Jinahn-gun, Jeollabuk-do				
<i>Zacco platypus</i>	40	24 (60.0)	1-1,823	206
<i>Zacco temminckii</i>	40	40 (100)	26-1,336	246
Subtotal	124	22 (17.7)	1-13	2.2
Geum-gang in Muju-gun, Jeollabuk-do				
<i>Zacco temminckii</i>	11	11 (100)	13-506	225
Geum-gang in Geumsan-gun				
<i>Zacco platypus</i>	38	38 (100)	101-11,805	3,375
<i>Zacco temminckii</i>	33	33 (100)	24-3,706	436
<i>Hemibarbus longirostris</i>	26	3 (11.5)	1-2	1.3
<i>Opsariichthys uncirostris</i>	4	4 (100)	12-562	255
<i>Pseudogobio esocinus</i>	6	1 (16.7)	-	1.0
<i>Acanthorhodeus gracilis</i>	1	1 (100)	-	21.0
Subtotal	124	22 (17.7)	1-13	2.2
Yugu-cheon in Gongju-si				
<i>Zacco platypus</i>	59	59 (100)	2,000-43,340	7,019
<i>Acheilognathus lanceolatus</i>	32	5 (15.6)	1-49	12.0
<i>Opsariichthys uncirostris</i>	18	18 (100)	182-5,240	871
<i>Acheilognathus rhombus</i>	15	1 (6.7)	-	4.0
<i>Hemibarbus longirostris</i>	7	2 (28.6)	1-2	1.5
Subtotal	131	85 (64.9)	1-43,340	5,057
Ji-cheon in Cheongyang-gun				
<i>Pseudogobio esocinus</i>	11	2 (18.2)	3-8	5.5
<i>Zacco temminckii</i>	29	29 (100)	7-509	98.6
<i>Acheilognathus koreensis</i>	10	10 (100)	27-456	192
<i>Zacco platypus</i>	4	4 (100)	128-1,637	754
Subtotal	43	43 (100)	7-1,637	181
Nonsan-cheon in Nonsan-si				
<i>Opsariichthys uncirostris</i>	7	2 (28.6)	1-2	1.5
Total	331	206 (62.2)	1-21,510	751

Geumsan-gun, Chungcheongnam-do. *Echinostoma* spp. metacercariae were detected in 19.7% fishes in positive fish species from 5 survey regions, and their mean intensity was about 10 per fish infected. However, the Korean dark sleepers, *O. platycephala*, from Yugu-cheon in Gongju-si were revealed 100% prevalence and about 48 metacercarial intensity. The metacer-

cariae of *C. complanatum* were found in fishes from only 2 survey regions, Yugu-cheon in Gongju-si and Geum-gang in Geumsan-gun, Chungcheongnam-do. Especially, their endemicity was relatively high in fishes from Yugu-cheon in Gongju-si, Chungcheongnam-do.

The fish collection is one of the important factors in the

Table 4. Infection status of *Echinostoma* spp. metacercariae (EsMc) in freshwater fish from Geum-gang in Jeollabuk-do and Chungcheongnam-do, Korea

Locality and fish sp.	No. of fish examined	No. (%) of fish infected	No. of EsMc detected	
			Range	Average
Juja-cheon in Jinahn-gun, Jeollabuk-do <i>Pungtungia herzi</i>	25	1 (4.0)	-	1.0
Geum-gang in Muju-gun, Jeollabuk-do <i>Pungtungia herzi</i>	35	5 (14.3)	1-2	1.2
Geum-gang in Geumsan-gun				
<i>Pseudogobio esocinus</i>	35	2 (5.7)	-	1.0
<i>Hemibarbus longirostris</i>	26	3 (11.5)	-	1.0
<i>Coreoperca herzi</i>	23	1 (4.3)	-	1.0
<i>Odontobutis platycephala</i>	22	8 (36.4)	1-13	3.5
<i>Sarcocheilichthys nigripinnis</i>	8	4 (50.0)	1-4	2.5
<i>Pungtungia herzi</i>	6	2 (33.3)	-	1.0
<i>Sarcocheilichthys variegatus</i>	3	1 (33.3)	-	1.0
<i>Acanthorhodeus gracilis</i>	1	1 (100)	-	1.0
Subtotal	124	22 (17.7)	1-13	2.2
Yugu-cheon in Gongju-si				
<i>Acheilognathus lanceolatus</i>	32	2 (6.3)	-	1.0
<i>Pungtungia herzi</i>	22	9 (40.9)	1-9	2.4
<i>Pseudogobio esocinus</i>	20	4 (20.0)	1-3	2.0
<i>Odontobutis platycephala</i>	10	10 (100)	5-123	47.7
<i>Sarcocheilichthys variegatus</i>	5	1 (20.0)	-	3.0
Subtotal	89	26 (29.2)	3-8	5.5
Ji-cheon in Cheongyang-gun <i>Pseudogobio esocinus</i>	11	2 (18.2)	3-8	5.5
Total	331	206 (62.2)	1-21,510	751

Table 5. Infection status of *Clinostomum complanatum* metacercariae (CcMc) in freshwater fish from Geum-gang in Chungcheongnam-do, Korea

Locality and fish sp.	No. of fish examined	No. (%) of fish infected	No. of CcMc detected	
			Range	Average
Geum-gang in Geumsan-gun				
<i>Squalidus japonicus coreanus</i>	5	2 (40.0)	-	1.0
<i>Acheilognathus yamatsutae</i>	7	3 (42.9)	-	1.0
<i>Hemibarbus longirostris</i>	6	1 (16.7)	-	1.0
<i>Hemibarbus labeo</i>	1	1 (100)	-	2.0
Subtotal	19	7 (36.8)	1-2	1.1
Yugu-cheon in Gongju-si				
<i>Acheilognathus lanceolatus</i>	72	62 (86.1)	1-64	1.3
<i>Hemibarbus longirostris</i>	32	2 (6.3)	-	2.0
<i>Acheilognathus yamatsutae</i>	20	19 (95.0)	1-61	18.1
<i>Pungtungia herzi</i>	17	5 (29.4)	1-18	4.6
<i>Carassius auratus</i>	10	2 (20.0)	4-27	15.5
<i>Squalidus gracilis majimae</i>	1	1 (100)	-	1.0
Subtotal	152	91 (59.9)	1-64	12.1
Total	171	98 (57.3)	1-64	11.3

metacercarial surveys for the trematode epidemiology. Total 1,161 fishes in 41 species from 6 survey regions in the water systems of Geum-gang were examined in this study. The

amount of fish samples was considerably different by the survey regions from 44 fishes in 11 species (Nonsan-cheon in Nonsan-si) to 386 fishes in 22 species (Geum-gang in Geum-

san-gun). Furthermore, 28 (68.3%) fish species were examined with below 20 in numbers, and the fish species examined with over 50 fish individuals was only 9 (22.0%) species, i.e., *Z. platypus* (n=141), *H. longirostris* (138), *P. esocinus* (136), *Z. temminckii* (113), *P. herzi* (101), *A. lanceolatus* (73), *C. herzi* (58), *O. platycephala* (57), and *C. auratus* (52). Accordingly, there are some differences in the fish collection by the survey regions and fish species in this study. These differences are unable to compare the infection status of ZTM by the survey regions in this study and previous studies. However, this study is able to reveal the general trends on the ZTM infections in fishes from the water systems of Geum-gang.

It has been known that the infection status of CsMc is showed a certain tendency in positive fish species from endemic sites, Wi-cheon in Gunwi-gun, Gyeongsangbuk-do and Yang-cheon in Sancheong-gun, Gyeongsangnam-do, according to the subfamily groups, i.e., Gobioninae, Acheilognathinae and Rasborinae, in the Cyprinidae fish hosts [11,12]. In this study, 30 (73.2%) fish species were the members of order Cypriniformes, and 26 (86.7%) out of 30 species were belonging to the family Cyprinidae (13 species in Gobioninae, 6 species in Acheilognathinae, 3 species in Rasborinae, 2 species in Cultrinae, 1 species in Leuciscinae and 1 species in Cyprininae) [22]. Among total 893 fish in family Cyprinidae, 454 (50.8%) ones were the members of Gobioninae, 147 (16.5%) were those of Acheilognathinae, 283 (31.7%) were those of Rasborinae, and 9 (1.0%) ones were those of other 3 subfamilies. A total of 122 (37.2%) out of 328 fish in 14 species, i.e., *P. herzi*, *S. variegatus*, *S. nigripinnis*, *S. japonicus coreanus*, *S. gracilis majimae*, *P. parva*, *P. esocinus*, *H. longirostris*, *H. eigenmanni*, *C. splendidus*, *A. yamatsutae*, *Z. platypus*, *O. uncirostris* and *S. scherzeri*, were infected with CsMc, and 119 (98.5%) fish in 13 (92.9%) species were the members of Cyprinidae. They all had been listed as the second intermediate hosts of *C. sinensis* in Korea [23,24]. On the other hand, the striped shinner, *P. herzi*, is to be known as an index fish species to check the endemicity of CsMc. Because of this fish species broadly lives in the water systems of Korea and is highly susceptible to CsMc. In this study, 52 (98.1%) out of 53 *P. herzi* (35 fish from Geum-gang in Mujugun, Jeollabuk-do, 15 ones from Geum-gang in Geumsan-gun and 3 ones from Ji-cheon in Cheongyang-gun, Chungcheongnam-do) were infected with 93.5 CsMc per fish infected, whereas 48 (25 fish from Juja-cheon in Jinahn-gun, Jeollabuk-do, 22 ones from Yugu-cheon in Gongju-si and only one from Nonsan-cheon in Nonsan-si, Chungcheongnam-do) out of 101 *P. herzi*

examined were negative with CsMc. The endemicities with CsMc were very low in other 13 fish species. Therefore, the infection tendency with CsMc could not show by the subfamily groups in the Cyprinidae fish hosts in this study.

The river basin of Geum-gang was known as the endemic area of metagonimiasis as well as clonorchiiasis [16-20]. It was also known that metagonimiasis is caused by *M. miyatai* and the main infection sources of this endemic disease are chubs, *Z. platypus* and *Z. temminckii*, in this region [16-18]. In this study, the endemicity of *Metagonimus* spp. metacercariae was not so high in 2 chub fish species. Prevalences were about 71% (100/141 *Z. platypus* examined) and 68% (77/113 *Z. temminckii* examined), but infection intensities were about 31 and 6 per fish infected. High prevalence and low burden with *Metagonimus* spp. metacercariae in chubs like in this study were also shown in most of rivers and streams in Gangwon-do. However, chubs from Joyang-gang (Jeongseon-gun) and Hantan-gang (Cheorwon-gun) revealed somewhat higher endemicities for *Metagonimus* spp. metacercariae [7,8].

The chub fish species, *Z. platypus*, *Z. koreanus* and *Z. temminckii*, are known to be the susceptible fish hosts for *C. armatus* metacercariae (CaMc) in Korea [23-25]. In this study, the high endemicities with CaMc were shown in *Z. platypus* from Geum-gang (100% prevalence and over 3,300 CaMc intensity) in Geumsan-gun and Yugu-cheon (100% prevalence and over 7,000 CaMc intensity) in Gongju-si, Chungcheongnam-do. In a nation-wide survey of CaMc [25], the prevalences were very high, 93.7-100% (av. 97.1%), in chub fish species, *Zacco* spp., from all surveyed sites. However, their infection intensities were more or less different by the surveyed areas and fish species. They were most high in Nakdong-gang in Gyeongsangnam-do (av., 4,201), and followed by Geum-gang (2,343), Nakdong-gang in Gyeongsangbuk-do (1,623), Han-gang (1,564), Tamjin-gang (1,550), Yeongsan-gang (1,493), streams in the east coast (1,028), Seomjin-gang (488) and Mangyeong-gang and Dongjin-gang (170). And then they were slightly higher in *Z. platypus* (av. 2,109) than in *Z. temminckii* (1,567) [25].

Infection status of *Echinostoma* spp. metacercariae (EsMc) was not so high in freshwater fish from Geum-gang in this study. However, it (100% prevalence and about 48 EsMc intensity) was relatively high in Korean dark sleeper, *O. platycephala*, from Yugu-cheon in Gongju-si, Chungcheongnam-do. Ahn et al. [26] detected a total of 32 *Echinostoma hortense* (= *Isthmiophora hortensis*) metacercariae (IhMc) in 10 (27.8%) out of 36

dark sleepers, *O. interrupta*, from Seom-gang in Wonseong-gun, Gangwon-do, Korea. Ahn and Ryang [27] found IhMc in 4 (22.2%) out of 18 dark sleepers from Namhan-gang in Gyeonggi-do and Gangwon-do. Lee et al. [28] detected IhMc in only 1 (2.3%) out of 44 *O. interrupta* from Cheongsong-gun, Gyeongsangbuk-do, Korea. Ryang [29] detected IhMc in 11 (20.3%) out of 54 dark sleepers from Chungju-ho (ho means lake) and the upper streams of Namhan-gang. Sohn et al. [8] reported relatively high prevalence (96.7%) and infection intensity (22 IhMc per fish infected) in 30 *O. platycephala* from Munsan-cheon in Paju-si, Gyeonggi-do. Recently, Sohn et al. [30] broadly surveyed the infection status with IhMc in dark sleepers, *Odontobutis* species, from some water systems of Korea. Infection status of IhMc in dark sleepers from Yugu-cheon was already included in Sohn et al. [30], and it was much higher than the findings of other previous studies [26-29].

In this study, *C. complanatum* metacercariae (CcMc) were detected in fishes from only 2 survey sites, Geum-gang in Geumsan-gun and Yugu-cheon in Gongju-si, Chungcheongnam-do. Overall prevalences in fishes from 2 sites were 36.8% and 59.9% in positive fish species, and intensities of infection were 1.1 and 12.1 per fish infected. Especially, in 2 species of bitterling, *Acheilognathus lanceolatus* and *A. yamatsutae*, from Yugu-cheon, prevalences were 86.1% and 95.0%, and intensities of infection were 11 and 18 per fish infected. Recently, Sohn et al. [31] broadly surveyed the infection status with CcMc in fishes from water systems of Nakdong-gang in Korea. In Sohn et al. [31], the infection tendency with CcMc was showed by the fish groups in fishes from Yang-cheon in Sancheong-gun Gyeongsangnam-do. The prevalence was most high in *Squalidus* spp. (97.7%) and followed by acheilognathinid fish (66.8%), *P. herzi* (52.0%), rasborinid fish (39.6%) and *Hemibarbus* spp. (25.9%). The intensity of infection was also most high in *Squalidus* spp. (av. 27 per fish infected) and followed by acheilognathinid fish (9), *P. herzi* (7), rasborinid fish (4), and *Hemibarbus* spp. (3). By the present study, 2 species of bitterling, *A. lanceolatus* and *A. yamatsutae*, from Yugu-cheon in Gongju-si, are to be heavily infected with CcMc as the members of acheilognathinid fish.

Conclusively, more than 5 species, i.e., *C. sinensis*, *Metagonimus* spp., *C. armatus*, *Echinostoma* spp. and *C. complanatum*, of ZTM were found in fishes from the water systems of Geum-gang in this study, but their overall endemicities were not so high. The endemicity of ZTM was variable and more or less different by the survey sites and fish species. The metacercariae

of *C. sinensis* were relatively endemic in striped shiner, *P. herzi*, from Geum-gang in Muju-gun, Jeollabuk-do and in Geumsan-gun, Chungcheongnam-do. The metacercariae of *C. armatus* were highly endemic in pale chub, *Z. platypus*, from Yugu-cheon in Gongju-si, and Geum-gang in Geumsan-gun, Chungcheongnam-do. Echinostome metacercariae were prevalent in Korean dark sleeper, *O. platycephala*, from Yugu-cheon in Gongju-si. The metacercariae of *C. complanatum* were relatively endemic in 2 species of bitterling, *A. lanceolatus* and *A. yamatsutae*, from Yugu-cheon in Gongju-si, Chungcheongnam-do.

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CONFLICT OF INTEREST

The authors have no conflicts of interest concerning the work reported in this paper.

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