



# Infection Characteristics of *Clonorchis sinensis* Metacercariae in Fish from Republic of Korea

Woon-Mok Sohn\*

Department of Parasitology and Tropical Medicine, and Institute of Health Sciences, Gyeongsang National University College of Medicine, Jinju 52727, Korea

**Abstract:** The infection nature of *Clonorchis sinensis* metacercariae (CsMc) in freshwater fish hosts is closely related to the transmission of human clonorchiasis. This article reviewed the infection characteristics of CsMc in freshwater fish in the Republic of Korea (Korea). The status of CsMc infection was examined in a total of 17,792 cyprinid fish of 49 species in 9 water systems, which included Hantan-/Imjin-gang, Han-gang, Geum-gang, Mangyeong-gang, Yeongsan-gang, Tamjin-gang, Seomjin-gang, Nakdong-gang, and streams in the east coastal areas from 2010 to 2020. The infection status of CsMc was examined according to cyprinid fish species and water systems, after which analyzed by endemicity and susceptibility index. The high endemicity was shown in the cyprinid fish from 3 regions (6.1%) in the upper reaches of Nakdong-gang, such as Banbyeon-cheon (stream), Yongjeon-cheon, and Wi-cheon. The moderate levels were observed in fishes from 8 regions (16.3%), and low endemicity was shown in fishes from 20 regions (40.8%). No CsMc were detected in fish from 18 regions (36.7%). The susceptibility of CsMc in index fish, *Puntungia herzi*, was found to be a reliable index without examination of other fish species. CsMc infection rates were closely related to subfamily groups in the cyprinid fish hosts in a highly endemic area. In Korea, a total of 58 fish species in 10 families has been listed as the second intermediate hosts for *C. sinensis*. This review provides several novel features of CsMc infection and clarifies the species of second intermediate freshwater fish host in Korea.

**Key words:** *Clonorchis sinensis* metacercariae, *Puntungia herzi*, second intermediate host, infection characteristics, endemicity, susceptibility index, index fish

## BACKGROUND AND PURPOSE

Until the 1970s, helminthic infections in humans had been highly prevalent in the Republic of Korea (Korea). Infections with the soil-transmitted helminths including *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms were the main helminthic diseases, but these helminthic infections have been drastically decreased by systematic nationwide control programs [1]. The soil-transmitted helminthiasis are no longer a health problem in Korea. However, helminthic infections associated with food-borne zoonotic trematodiasis including *Clonorchis sinensis* continue to occur in the riverside areas. The oriental liver fluke *C. sinensis* is currently the most important helminth in terms of prevalence and clinical significance. The endemicity of clonorchiasis has maintained at relatively high levels, especially in the riverside regions in Korea [1-7]. An epi-

demiological survey conducted from May 1979 to April 1980 reported a high prevalence of clonorchiasis in the riverside residents of 7 major rivers, such as Nakdong-gang (gang means river) (40.2%), Yeongsan-gang (30.8%), Seomjin-gang (17.3%), Tamjin-gang (15.9%), Han-gang (15.7%), Geum-gang (12.0%), and Mangyeong-gang (8.0%) [2]. In a survey conducted in 2006, the prevalence of clonorchiasis among residents living in the Nakdong-gang, Seomjin-gang, Yeongsan-gang, and Geum-gang basins was 17.1%, 11.2%, 5.5%, and 4.6%, respectively [3]. Another study done in 2008 also observed the prevalence of clonorchiasis among residents of 5 major rivers: Nakdong-gang, Seomjin-gang, Geum-gang, Yeongsan-gang, and Han-gang was 11.7%, 9.9%, 6.5%, 3.1%, and 1.0%, respectively [6]. In a nationwide survey on helminthic infection in Korea [1], the prevalence of clonorchiasis was the highest. Approximately 932,540 people (1.9%) were estimated to be infected with *C. sinensis*. A recent study observed 5.3% prevalence of clonorchiasis in the residents of Haman-gun, Gyeongsangnam-do, which is located in the Nakdong-gang basin [7]. Although the endemicity of clonorchiasis is decreasing, this endemic trematodiasis still remains a major public health problem to be addressed in this country.

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\*Corresponding author (wmsohn@gnu.ac.kr)

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Since the first report of *C. sinensis* metacercariae (CsMc) by Kobayashi in Korea, many Korean parasitologists, epidemiologists, and public health workers have surveyed and estimated the endemicity of clonorchiasis through investigations of freshwater fishes, the sources of human infection [8-31]. In order to determine the status of CsMc infection, freshwater fishes from 34 different localities were examined [19]. We also investigated the infection status of zoonotic trematode metacercariae (ZTM) including CsMc in fish from various water systems of Korea [20-31]. The infection status of CsMc in freshwater fish from 3 wide regions, which were tentatively divided by the latitudinal levels of Korean peninsula were surveyed [20]. We also surveyed on the prevalence of ZTM in freshwater fish from Gangwon-do (Province) [21]. We 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 located in relatively northern parts of Korea [22]. The prevalence of CsMc in freshwater fishes from the water systems of Seomjin-gang and Tamjin-gang were also investigated [20,24]. We recently reported the prevalence and infection intensity of CsMc in freshwater fish from highly endemic focus, Wi-cheon and Yongjeon-cheon (branch streams of Nakdong-gang), in Gunwi-gun and Cheongsong-gun, Gyeongsangbuk-do, Korea [25,26]. Freshwater fishes from Geum-gang, Soyang-cheon (branch stream of Mangyeong-gang in Wanju-gun, Jeollabuk-do), Yang-cheon and Deok-cheon-gang (in Sancheong-gun, Gyeongsangnam-do), and Togyo-jeosuji (in Cheorwon-gun, Gangwon-do) were also examined to observe the infection status of CsMc [27-31].

There are several Korean and Chinese review articles on the biology of *Clonorchis sinensis* and pathogenetic factors, histopathological features, and chemotherapy for clonorchiasis [32-37]. Especially, Rim [32] reviewed on the pathobiology and chemotherapy of clonorchiasis in Korea, and other workers have also investigated multifarious contents of clonorchiasis in Korea, China, and other countries [33-37]. However, the status and characteristics of CsMc infections in fish intermediate hosts have not been systematically and extensively analyzed in Korea. This article reviewed data from published and unpublished results obtained from studies on the detection of metacercaria by localities and by fishes in our laboratory over the past several years. The status of CsMc infection was analyzed on a total of 17,792 cyprinid fish of 49 species among all fishes collected from 9 main water systems in Korea, which included Hantan-/Imjin-gang, Han-gang, Geum-gang, Mangyeong-gang, Yeong-

san-gang, Tamjin-gang, Seomjin-gang, Nakdong-gang, and streams in the east coastal areas.

## DATA COLLECTION AND ANALYSIS

All fishes collected were placed on ice and transported to the laboratory of the Department of Parasitology and Tropical Medicine, Gyeongsang National University College of Medicine, Jinju, Korea. The fish species was identified, after which individual fish was finely ground in a mortar with pestle. The ground fish meat was mixed with artificial gastric juice, and incubated at 37°C for about 2 hr. The digested material was filtered through a mesh (pore size 1×1 mm) and washed with physiological saline until the supernatant became clear. The sediment was carefully examined under a stereomicroscope. CsMcs were separately collected according to previously described method [38]. CsMcs were counted to determine the infection rate (No. of fish with CsMc/No. of fish examined×100) and intensity (No. of CsMc/fish infected) by fish species. The susceptibility index (SI) of CsMc in each fish species was calculated by the formula, prevalence/100×mean metacercarial intensity per fish infected (PFI). The endemicity was determined by the formula, positive rate (No. of positive species/No. of cyprinid fish spp. examined) of fish spp.×positive rate (No. of positive fish/No. of cyprinid fish examined) in positive cyprinid fish spp.×mean No. of CsMc detected. The endemicity was categorized into 4 groups, i.e., negative (no CsMc), low (below 10), moderate (10.01-100), and high (over 100.01) by the endemic index.

## SURVEYED AREAS

The overall cyprinid fishes examined by the survey localities are presented in Table 1 and each survey locality is presented in Fig. 1.

In the water systems of Hantan-/Imjin-gang, a total of 1,954 cyprinid fishes (34 species) collected in 8 local sites, i.e., ① Hantan-gang in Cheorwon-gun (Latitude: 38.23047; Longitude: 127.2179) and ② Hantan-gang in Yeoncheon-gun (37.94375; 127.07142), ③ Namdae-cheon (cheon means stream) in Cheorwon-gun (38.43268; 127.4375), Gangwon-do, ④ Chatan-cheon in Yeoncheon-gun (38.0855; 127.07264) and ⑤ Yeongpyeong-cheon in Pocheon-si (38.01408; 127.2088), ⑥ Imjin-gang in Yeoncheon-gun (38.04905; 127.02154), ⑦ Munsan-cheon in Paju-si (37.80837; 126.85643), Gyeonggi-do, and ⑧ Togyo-

**Table 1.** Summary of cyprinid fishes<sup>a</sup> examined by the survey localities

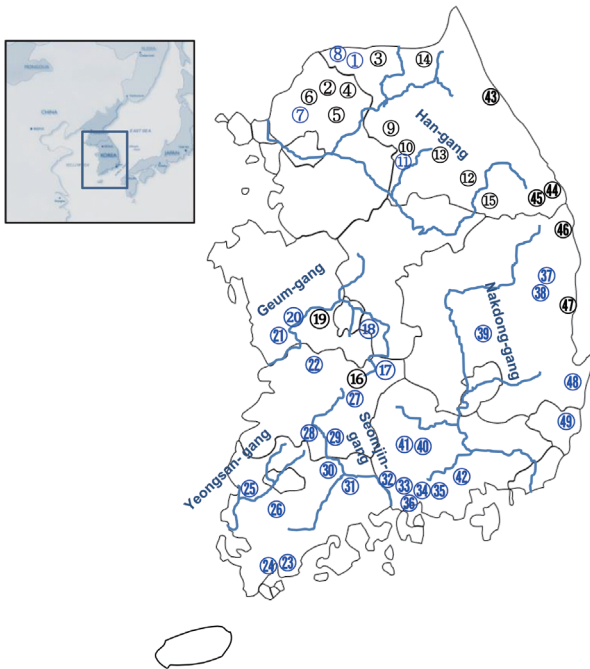
Locality No. & water system	Administrative region	No. of fish spp. examined	No. (NIF) <sup>b</sup> of cyprinid fish examined
① Hantan-gang	Cheorwon-gun, Gangwon	26	888 (137)
② Hantan-gang	Yeoncheon-gun, Gyeonggi	7	35 (3)
③ Namdae-cheon	Cheorwon-gun	8	105 (13)
④ Chatan-cheon	Yeoncheon-gun	8	106 (25)
⑤ Yeongpyeong-cheon	Pocheon-si, Gyeonggi	6	54 (0)
⑥ Imjin-gang	Yeoncheon-gun	8	123 (2)
⑦ Munsan-cheon	Paju-si, Gyeonggi	8	130 (0)
⑧ Togyo-jeosuji	Cheorwon-gun	15	513 (16)
⑨ Hongcheon-gang	Hongcheon-gun, Gangwon	11	138 (25)
⑩ Seom-gang	Hoengseong-gun, Gangwon	13	171 (14)
⑪ Seom-gang	Wonju-si, Gangwon	20	643 (121)
⑫ Joyang-gang	Jeongseon-gun, Gangwon	10	138 (24)
⑬ Pyeongchang-gang	Pyeongchang-gun, Gangwon	12	198 (35)
⑭ Sooip-cheon	Yanggu-gun, Gangwon	12	169 (11)
⑮ Dong-gang	Yeongweol-gun, Gangwon	12	211 (16)
⑯ Juja-cheon	Jinan-gun, Jeonbuk	11	168 (25)
⑰ Geum-gang	Muju-gun, Jeonbuk	8	65 (35)
⑱ Geum-gang	Geumsan-gun, Chungnam	17	307 (15)
⑲ Yugu-cheon	Gongju-si, Chungnam	12	281 (22)
⑳ Ji-cheon	Cheongyang-gun, Chungnam	8	74 (3)
㉑ Nonsan-cheon	Nonsan-si, Chungnam	8	29 (1)
㉒-1.Soyang-cheon	Wanju-gun, Jeonbuk	12	345 (98)
㉒-2.Soyang-cheon	Wanju-gun	14	343 (87)
㉓-1.Tamjin-gang	Jangheung-gun, Jeonnam	16	918 (152)
㉓-2.Tamjin-gang	Jangheung-gun	16	441 (57)
㉔ Tamjin-gang	Gangjin-gun, Jeonnam	11	420 (70)
㉕ Jiseok-cheon	Naju-si, Jeonnam	9	86 (0)
㉖ Yeongam-cheon	Yeongam-gun, Jeonnam	8	45 (2)
㉗ Osu-cheon	Imsil-gun, Jeonbuk	11	236 (0)
㉘-1.Seomjin-gang	Sunchang-gun, Jeonbuk	22	268 (56)
㉘-2.Seomjin-gang	Sunchang-gun	26	612 (104)
㉙ Songdae-cheon	Namwon-si, Jeonbuk	19	396 (83)
㉚-1.Seomjin-gang	Gokseong-gun, Jeonnam	13	275 (22)
㉚-2.Seomjin-gang	Gokseong-gun	17	582 (44)
㉛ Seomjin-gang	Gurye-gun, Jeonnam	21	322 (33)
㉜ Hwagye-cheon	Hadong-gun, Gyeongnam	12	179 (17)
㉝ Akyang-cheon	Hadong-gun	7	145 (40)
㉞ Namsan-cheon	Hadong-gun	12	322 (37)
㉟ Hoeng-cheon	Hadong-gun	11	281 (42)
㊱ Jugyo-cheon	Hadong-gun	16	174 (2)
㊲ Banbyeon-cheon	Yeongyang-gun, Gyeongbuk	10	155 (26)
㊳ Yongjeon-cheon	Cheongsong-gun, Gyeongbuk	15	634 (234)
㊴-1.Wi-cheon	Gunwi-gun, Gyeongbuk	19	501 (72)
㊴-2.Wi-cheon	Gunwi-gun	21	592 (97)
㊴-3.Wi-cheon	Gunwi-gun	23	715 (105)
㊵-1.Yang-cheon	Sancheong-gun, Gyeongnam	19	1,260 (408)
㊵-2.Yang-cheon	Sancheong-gun	18	978 (138)
㊶ Deokcheon-gang	Sancheong-gun	13	738 (123)
㊷ Jisu-cheon <sup>c</sup>	Jinju-si, Gyeongnam	11	164 (1)
㊸ Namdae-cheon	Yangyang-gun, Gangwon	7	194 (31)

(Continued to the next page)

**Table 1.** Continued

Locality No. & water system	Administrative region	No. of fish spp. examined	No. (NIF) <sup>b</sup> of cyprinid fish examined
44 Osip-cheon	Samcheok-si, Gangwon	8	168 (6)
45 Gagok-cheon	Samcheok-si, Gangwon	5	89 (17)
46 Whangpi-cheon	Ulsin-gun, Gyeongbuk	8	167 (47)
47 Osip-cheon	Yeongdeok-gun, Gyeongbuk	10	179 (43)
48 Gigye-cheon	Gyeongju-si, Gyeongbuk	9	87 (18)
49 Taehwa-gang	Ulsan Metropolitan City	10	235 (48)

cheon, small river; gang, river; gun, County; si, City.  
<sup>a</sup>Total 17,792 (2,903) cyprinid fish in 49 species were examined.  
<sup>b</sup>(NIF): No. of index fish *P. herzi* examined.  
<sup>c</sup>fish from Haman-cheon in Haman-gun, Gyeongsangnam-do, Korea.



**Fig. 1.** A total of 49 survey localities marked in this map is subjected to analyze in this review. Detailed information of each survey locality (Number of water system and administrative region) is shown in Table 1.

jeosuji (reservoir lake) in Cheorwon-gun (38.27082; 127.28949), Gangwon-do, Korea were examined for the CsMc. The number and species of fish (year) examined are summarized in detail in Supplementary Table S1.

A total of 1,668 cyprinid fishes (29 spp.) were examined in 9 localities of Han-gang, i.e., 9 Hongcheon-gang in Hongcheon-gun (37.76568; 127.97006), 10 Seom-gang in Hoengseong-gun (37.50058; 127.99337), 11 Seom-gang in Wonju-si (37.42690; 127.89634), 12 Joyang-gang in Jeongseon-gun (37.44292; 128.66256), 13 Pyeongchang-gang in Pyeongchang-gun (37.32968; 128.37765), 14 Sooip-cheon in Yanggu-gun (38.20845;

127.94321), and 15 Dong-gang in Yeongweol-gun (37.18663; 128.48415), Gangwon-do. The number and species of fish (year) examined are presented in detail in Supplementary Table S2.

In the water systems of Geum-gang, a total of 924 cyprinid fishes (26 spp.) were examined in 6 localities, i.e., 16 Juja-cheon in Jinan-gun (35.98023; 127.39388), 17 Geum-gang in Muju-gun (35.97529; 127.55662), Jeollabuk-do, 18 Geum-gang in Geumsan-gun (36.11427; 127.58775), 19 Yugu-cheon in Gongju-si (36.53727; 126.94847), 20 Ji-cheon in Cheongyang-gun (36.38958; 126.85174), and 21 Nonsan-cheon in Nonsan-si (36.19906; 127.06790), Chungcheongnam-do, Korea. The number and species of fish (year) examined were shown in detail in Supplementary Table S3. Total 2,598 cyprinid fishes (27 spp.) were examined in the water systems of Mangyeong-gang (22 Soyang-cheon) in Wanju-gun (36.11427; 127.58775), Jeollabuk-do, 23 Tamjin-gang in Jangheung-gun (34.42572; 126.54322) and 24 Tamjin-gang in Gangjin-gun (34.38053; 126.48514), Jeollanam-do, Yeongsan-gang (25 Jiseok-cheon) in Naju-si (35.04768; 126.80448) and (26 Yeongam-cheon) in Yeongam-gun (35.04086; 126.65664), Jeollanam-do. The number and species of fish examined by year are summarized in detail in Supplementary Table S4.

A total of 2,691 cyprinid fishes (36 spp.) were examined in the water systems of Seomjin-gang i.e., 27 Osu-cheon in Imsil-gun (35.52768; 127.32885), 28 Seomjin-gang in Sunchang-gun (35.43854; 127.24047), 29 Songdae-cheon in Namwon-si (35.91616; 127.15413), 30 Seomjin-gang in Gokseong-gun (35.14903; 127.32589), and 31 Seomjin-gang in Gurye-gun (35.14340; 127.31661), in Jeollabuk-do and Jeollanam-do. The number and species of fish (year) examined were designated in detail in Supplementary Table S5. In the water systems of Seomjin-gang in Hadong-gun, Gyeongsangnam-do,



total 1,101 cyprinid fishes (24 spp.) were examined in 5 localities, i.e., ㉓ Hwagye-cheon (35.02828; 127.81974), ㉔ Akyang-cheon (35.16218; 127.71133), ㉕ Namsan-cheon (35.09540; 127.79806), ㉖ Hoeng-cheon (35.10716; 127.80779), and ㉗ Jugyo-cheon (35.02828; 127.81974). The number and species of fish (year) examined are shown in Supplementary Table S6 in detail.

A total of 5,737 cyprinid fishes (29 spp.) were examined in 6 localities, i.e., ㉘ Banbyeon-cheon in Yeongyang-gun (36.59338; 129.06975), ㉙ Yongjeon-cheon in Cheongsong-gun (36.40716; 129.36594), ㉚ Wi-cheon in Gunwi-gun (36.18863; 128.64873), ㉛ Yang-cheon in Sancheong-gun (35.36021; 128.05820), ㉜ Deokcheon-gang in Sancheong-gun (35.24643; 127.89224), ㉝ Jisu-cheon in Jinju-si (35.33582; 128.32520), and Haman-cheon in Haman-gun (35.20562; 128.44302), of Nakdong-gang in Gyeongsangbuk-do and Gyeongsangnam-do. The number and species of fish (year) examined are shown in Supplementary Table S7 in detail.

In the streams in the east coastal areas, i.e., ㉞ Namdae-cheon in Yangyang-gun (38.07302; 128.59303), ㉟ Osip-cheon in Samcheok-si (37.42217; 129.11746), ㊱ Gagok-cheon in Samcheok-si (37.14106; 129.29423), Gangwon-do, ㊲ Whangpi-cheon in Uljin-gun (36.96583; 129.39499), ㊳ Osip-cheon in Yeongdeok-gun (36.40716; 129.36594), ㊴ Gygae-cheon in Gyeongju-si (36.03105; 129.24680), Gyeongsangbuk-do and ㊵ Taehwa-gang (35.58515; 129.22520), and Cheokgwa-cheon (35.59894; 129.27461) in Ulsan Metropolitan City, a total of 1,119 cyprinid fishes (20 spp.) were examined. The number and species of fish (year) examined are summarized in detail in Supplementary Table S8.

## INFECTION STATUS OF CsMc IN CYPRINID FISH BY SURVEY REGIONS AND FISH SPECIES

### Infection status of fish in Hantan-/Imjin-gang and Han-gang

CsMcs (Fig. 2) were detected in cyprinid fish from 3 (Hantan-gang and Togyo-jeosuji in Cheorwon-gun and Munsan-cheon in Paju-si) out of 8 localities in the water systems of Hantan-gang and Imjin-gang. Their infection rates were 8.9%, 40.0%, and 43.8%, respectively. The infection intensities were 2.2, 35.6 and 37.8 PFI, respectively. In the water systems of Han-gang, CsMcs were found only in cyprinid fish from Seom-gang in Wonju-si, Gangwon-do. The prevalence and infection intensity were very low, 5.4% and 1.6 PFI, respectively. The infection



**Fig. 2.** Three metacercariae of *C. sinensis* detected in a striped shinner, *Puntungia herzi*, from Wi-cheon in Gunwi-gun, Gyeongsangbuk-do, Korea. They were elliptical and 145-172 × 125-158 μm in size (average 160 × 140 μm), had nearly equal sized 2 suckers, brownish pigment granules, and an O-shaped excretory bladder. Scale bar is 50 μm.

status by the fish species and locality is presented in Supplementary Table S9. No CsMc was detected in fish from 6 localities, which included Hongcheon-gang, Seom-gang in Hoengseong-gun, Joyang-gang, Pyeongchang-gang, Sooip-cheon, and Dong-gang of Han-gang in Gangwon-do.

A previous epidemiological survey on the infections of intestinal helminths in residents and those of ZTM in fishes in riverside areas of Hantan-gang in Cheorwon-gun, Gangwon-do detected the eggs of *C. sinensis* in 39 (8.4%) out of 465 fecal samples examined, while they could not find CsMc in 68 fish (13 species) including 10 *P. herzi* [39]. However, CsMcs were consecutively detected in fishes from Hantan-gang, Munsan-cheon, and Togyo-jeosuji, which are located in the northern parts of Korea [20-22,31]. Among localities where CsMcs were found, Togyo-jeosuji, a lake for agricultural water supply, is located within Civilian Control Line in Dongsong-eup (township), Cheorwon-gun. On the other hand, CsMc had not been detected in all fishes examined in the water systems of Han-gang in Gangwon-do before 2018 [20,21]. Interestingly, however, 3 fish species, i.e., *P. herzi*, *P. parva*, and *S. variegatus wakiyae*, thrive in Seom-gang in Wonju-si, Gangwon-do, were found to be infected with CsMc although their prevalence and infection intensity were very low.

### Infection status in fish from Geum-gang

Several epidemiological studies have investigated the infection status of zoonotic trematodes including *C. sinensis* of the residents in the riverside areas of Geum-gang [40-43], while

those on the infections of CsMc in fish hosts have scarcely been done. Recently, Sohn et al. [27] extensively surveyed the infection status of ZTM in fish from 6 riverside areas of Geum-gang. They detected CsMc in 119 out of 316 cyprinid fishes (37.7%) in the positive fish species (PFS) from 4 surveyed areas, such as Geum-gang (Muju-gun, Jeollabuk-do and Geumsan-gun, Chungcheongnam-do), Ji-chen (Cheongyang-gun, Chungcheongnam-do), and Nonsan-cheon (Nonsan-si, Chungcheongnam-do). Their mean infection intensity was 43.9 PFI. No CsMc was detected in fish from 2 localities, Jujacheon (Jinan-gun, Jeollabuk-do) and Yugu-cheon (Gongju-si, Chungcheongnam-do) [27]. The infection status by the fish species and by surveyed areas is depicted in detail in Supplementary Table S10.

#### Infection status of fish from Mangyeong-gang

In the early 1980s, epidemiological studies had been performed to investigate the infection status of CsMc and DTM in fishes from Mangyeong-gang [14]. Soyang-cheon, located in Wanju-gun, Jeollabuk-do, is a branch stream of Mangyeong-gang. Recently, Sohn et al. [28] investigated the infection status of ZTM in freshwater fishes in this area for 2 times during 2013-2015 and 2018-2019, respectively. They detected CsMc in 205 (35.4%) out of 579 cyprinid fishes in PFS. The infection intensity was 114.6 PFI. The prevalence and mean intensity were different from each other; prevalence of 48.1% and 21.8%, and mean intensities of 22.2 and 235.0 PFI, in the first survey and the second survey, respectively [28]. The infection status of CsMc by the fish species and by survey period is shown in Supplementary Table S11.

There was an obvious discrepancy between the prevalence and CsMc infection intensity in 2 consecutive surveys. The endemicity of CsMc in 2 highly susceptible species of fish, *P. herzi* and *S. variegatus wakiye*, were relatively high in latter period, especially in 2019 [28]. They observed 31.4% prevalence from 659 fishes in 14 PFS and 114 PFI infection intensity. However, Rhee et al. [14] reported 59.2% prevalence and 42.0 CsMc PFI infection intensity in 157 fishes in 12 PFS from Mangyeong-gang. The prevalence of CsMc was high by Rhee et al. [14], while infection intensity was high by Sohn et al. [28]. In Rhee et al. [14], false dace, *Pseudorasbora parva*, was the most prevalent (96.8%) with the highest intensity of infection (119 CsMc PFI). Meanwhile, 4 out of 8 *P. herzi* examined were infected with 5 CsMc in average. At that time, *P. parva* was the most susceptible fish host and regarded as the index fish host for clo-

norchiasis epidemiology. However, in a recent study, Sohn et al. [28] could not examine the infection status of CsMc in *P. parva*, but they found that striped shinner, *P. herzi*, was the most dominant species and could be an index fish host for clonorchiasis epidemiology.

#### Infection status in fish from Tamjin-gang

Tamjin-gang is one of the major rivers in Jeollanam-do together with Seomjin-gang and Yeongsan-gang, and the riverside areas of this river has been known as the endemic area of intestinal fluke, *Metagonimus yokogawai* and heterophyid flukes [2,44-48]. Yoon et al [24] widely and systematically examined the infection status with CsMc in fish in this river. They detected CsMc in 625 (51.1%) out of 1,224 cyprinid fishes in PFS from Tamjin-gang in Jangheung-gun and Ganjin-gun, Jeollanam-do. Their mean intensity of infection was 50.9 PFI [24]. In fish from Jangheung-gun, prevalence was 49.4% and 45.1%, and mean infection intensities were 51.5 and 42.4 PFI during the former (2014-2017) and the latter (2018-2019) survey periods. They also found CsMc in 161 (62.7%) out of 257 fishes in 8 PFS from the lower reaches of Tamjin-gang in Gangjin-gun, Jeollanam-do, and their mean intensity of infection was 57.2 PFI [24]. The infection status of CsMc by the fish species and by survey localities (years) is shown in Supplementary Table S12.

CsMcs are more or less prevalent in fish from Tamjin-gang [24]. In the PFS group, the endemicity of CsMc was slightly higher in fish from the lower reaches in Gangjin-gun (prevalence: 62.7%; infection intensity: 57.2 PFI) than in fish from the middle reaches in Jangheung-gun (48.0%; 48.7 PFI). On the other hand, Cho et al. [20] reported 76.5% prevalence and infection intensity of 206 CsMc PFI in 51 fishes from Tamjin-gang in Gangjin-gun, but no CsMc was found in 52 fishes collected from Jangheung-gun. These collective data suggest that the endemicity of CsMc has been continuously maintained in fish from Gangjin-gun. However, no CsMc was found in fish from Jangheung-gun remains with uncertainty [20].

#### Infection status in fish from Yeongsan-gang

Epidemiological study on the infection status of CsMc in fish from Yeongsan-gang has been poorly understood. A previous study detected CsMc in 23 (35.4%) out of 65 freshwater fish from Yeongsan-gang in Naju-si, Jeollanam-do with the infection intensity of 79.6 PFI [20]. Recently, we detected CsMc in 41 (61.2%) out of 67 cyprinid fishes in PFS from 2 localities

of Yeongsan-gang (Jiseok-cheon in Naju-si and Yeongam-cheon in Yeongam-gun, Jeollanam-do). Their mean infection intensity was 6.6 PFI. The prevalence was 68.8% and 42.1% with the infection intensities of 6.6 and 6.8 PFI, respectively. The infection status with CsMc by the fish species and by surveyed areas is presented in Supplementary Table S13.

#### Infection status in fish from Seomjin-gang

Cho et al. [20] investigated the infection status of CsMc in freshwater fish from 3 wide regions. They tentatively divided Korean peninsula by the latitudinal levels. However, they included the fishes only from 2 sites of Seomjin-gang, Gokseong-gun (191 fish in 22 spp.) and Gurye-gun (68 fish in 14 spp.), Jeollanam-do. Kim et al. [19] surveyed a total of 677 freshwater fishes (21 spp.) from 34 localities to observe the infection status with CsMc in Korea, while they examined 29 fishes (4 spp.) and 45 fishes (10 spp.) from only 2 sites in Seomjin-gang in Imsil-gun (Jeollabuk-do) and in Gokseong-gun (Jeollanam-do). However, the epidemiological study on the infection status of CsMc in fish from Seomjin-gang has not been widely and systematically performed.

Sohn et al. [23] examined a total of 1,604 freshwater fishes from 7 local sites of Seomjin-gang basins; i.e., Osu-cheon (Imsil-gun), upper reaches of Seomjin-gang (Sunchang-gun), and Songdae-cheon (Namwon-si) in Jeollabuk-do, middle reaches of Seomjin-gang (Gokseong-gun and Gurye-gun in Jeollanam-do), and lower reaches of Seomjin-gang (Hoeng-cheon and Namsan-cheon) in Hadong-gun, Gyeongsangnam-do, for 5 years (2012-2016). We analyzed the unpublished epidemiological data (2017-2020) on the infection status of CsMc in fish from Seomjin-gang together with previously published data [23].

Our team detected CsMc in 444 (47.4%) out of 936 cyprinid fishes in PFS from the upper reaches of Seomjin-gang in Jeollabuk-do (Imsil-gun, Sunchang-gun, and Namwon-si). Their mean infection intensity was 12.6 PFI. The prevalence was 22.3%, 63.9%, 44.1%, and 66.1% with infection intensities of 4.4, 11.1, 16.1, and 9.5 PFI, respectively, in fish from Osu-cheon, Seomjin-gang (2014-2015), and Seomjin-gang (2018-2020) in Sunchang-gun and Songdae-cheon. Supplementary Table S14 summarizes the infection status of CsMc by fish species, surveyed area, and survey year.

We also observed CsMc in 369 (43.3%) out of 853 cyprinid fishes in PFS from the middle reaches of Seomjin-gang in Jeollanam-do. Their mean infection intensity was 22.0 PFI. The

prevalence was 45.2%, 36.3%, and 55.6% and infection intensities were 22.5, 6.8, and 42.1 PFI, respectively, in fish from Gokseong-gun (2015-2016), Gokseong-gun (2018-2020), and Gurye-gun. The infection status of CsMc by fish species, surveyed area, and survey year is summarized in Supplementary Table S15.

Our team recently detected CsMc in 26 (14.0%) out of 186 cyprinid fishes in PFS from the lower reaches of Seomjin-gang in Hadong-gun, Gyeongsangnam-do (Hwagye-cheon, Akyang-cheon, Namsan-cheon, Hoeng-cheon, and Jugyo-cheon). Their mean infection intensity was 21.8 PFI. Prevalence in each region was 39.5%, 14.3%, 8.1%, 2.2%, and 38.5% with infection intensities of 6.1, 1.0, 113.0, 1.0, and 26.8 PFI, respectively. Supplementary Table S16 shows an infection status of CsMc by the fish species and by surveyed areas.

These collective data demonstrated that the endemicity of CsMc is relatively low in fish from the lower (Hadong-gun, Gyeongsangnam-do) and upper reaches (Imsil-gun, Sunchang-gun, and Namwon-si, Jeollabuk-do) of Seomjin-gang compared with that in fish from the middle reaches of Seomjin-gang (Gokseong-gun and Gurye-gun in Jeollanam-do) [23]. The infection rates and intensities of CsMcs were not so high in PFS from Imsil-gun and Gokseong-gun [19]. The prevalence (35.3% and 66.1%) and infection intensities (59.4 and 37.6 PFI) in fish from Gokseong-gun and Gurye-gun (Jeollanam-do) [20] were similar or slightly higher than those observed by our study [23].

#### Infection status in fish from Nakdong-gang

We identified CsMc in 474 (63.2%) out of 750 cyprinid fishes in PFS from upper reaches of Nakdong-gang in Gyeongsangbuk-do (Banbyun-cheon in Yeongyang-gun and Yongjeon-cheon in Cheongsong-gun). The mean infection intensity was 572.3 PFI. Prevalence in each survey region was 81.6% and 59.5% with the infection intensities of 190.1 and 677.1 [26]. Supplementary Table S17 summarizes the infection status by the fish species and by survey sites. Sohn et al. [25] also found CsMc in 1,172 (65.6%) out of 1,787 cyprinid fishes in PFS from Wi-cheon in Gunwi-gun with mean infection intensity of 453.0 PFI. Prevalence in each survey year was 63.5% (2011, 2013, and 2014), 70.8% (2015-2017) and 62.8% (2018-2020) with infection intensities of 716, 585, and 148, respectively [25]. The infection status by fish species and surveyed year is shown in Supplementary Table S18.

Three streams, Banbyeon-cheon, Yongjeon-cheon, and Wi-

cheon, are the branches of Nakdong-gang, which are located in Yeongyang-gun, Cheongsong-gun, and Gunwi-gun, Gyeongsangbuk-do. These streams flow to the inland of Gyeongsangbuk-do, where upper Nakdong-gang flows. Infection status of CsMc in fishes thrive in these streams is high. Prevalence in each stream was 81.6%, 59.5%, and 65.6% with infection intensities of 190.1, 677.1, and 453.0 PFI, respectively. The SI was also the highest in fish from Yongjeon-cheon (402.9) followed by Wi-cheon (297.2) and Banbyeon-cheon (155.1) [25,26]. Interestingly, the SI of Wi-cheon showed a tendency to gradually decrease along with the surveyed period, 454.7 (2011, 2013, 2014), 414.2 (2015-2017), and 92.9 (2018-2020), respectively [25].

Sohn et al. [29] observed CsMc in 1,293 (61.9%) out of 2,088 cyprinid fishes in PFS from Yang-cheon (Sancheong-gun, Gyeongsangnam-do). Their mean infection intensity was 82.1 PFI. Prevalence and infection intensity were different each other according to surveyed periods: 63.2% with 96.2 PFI (2011-2014) and 60.0% with 60.3 PFI (2015-2017), respectively. The infection status by the fish species and by surveyed period is shown in Supplementary Table S19.

Our team also detected CsMc in 254 (35.9%) out of 708 cyprinid fishes in PFS from Deokcheon-gang (Sancheong-gun), Jisu-cheon (Jinju-si), and Haman-cheon (Haman-gun) in Gyeongsangnam-do. Their mean infection intensity was 25.4 PFI [30]. Prevalence in each survey region was 39.0% and 21.0%, and infection intensities were 27.6 and 6.5, respectively. The infection status by the fish species and by surveyed regions is presented in Supplementary Table S20.

The riverside areas of Gyeongho-gang such as Yang-cheon and Deokcheon-gang (Sancheong-gun, Gyeongsangnam-do), and Nam-gang (the lower reaches of Nakdong-gang located in Gyeongsangnam-do), have been reported as high endemic areas of clonorchiasis [18,49-51]. However, the infection status of CsMc in fish from these areas was not high [29,30]. SI was 50.8, 10.8, and 1.4 in fish from Yang-cheon, Deokcheon-gang, Jisu-cheon (a branch of Nam-gang in Jinju-si) and Haman-cheon (another branch of Nam-gang in Haman-gun), respectively. In case of Yang-cheon, the SI showed a tendency to decrease from 60.8 (2011-2014) to 36.2 (2015-2017) [29].

#### Infection status in fish from streams in east coastal areas

Our team detected CsMc in 86 (55.8%) out of 154 cyprinid fishes in PFS from Gigyae-cheon in Gyeongju-si, Gyeongsangbuk-do and Cheokgwa-cheon and Taehwa-gang in Ulsan Me-

tropolitan City. Their mean infection intensity was 15.7 PFI. Prevalence in each survey region was 72.2% and 53.7% with infection intensities of 5.2 and 17.5 PFI, respectively. No CsMc was detected in fish from 3 local streams in Gangwon-do (Namdae-cheon, Osip-cheon, and Gagok-cheon) and 2 local streams in Gyeongsangbuk-do (Whangpi-cheon and Osip-cheon). The infection status by the fish species and by surveyed regions is shown in Supplementary Table S21.

Table 2 summarizes the overall infection status of CsMc in cyprinid fish by the water systems of Korea.

### ENDEMICITY OF CsMc IN CYPRINID FISH BY SURVEY AREAS

The endemicity of CsMc in cyprinid fish from ① Hantangan and ⑪ Seom-gang was low (0.05 and 0.01), while that from ⑦ Munsan-cheon and ⑧ Togyo-jeosuji was moderate (12.5 and 12.1). In the water systems of Geum-gang, the endemicity was low in fish from ⑱ Geum-gang in Geumsan-gun (4.3), ⑳ Ji-cheon (4.0), and ㉑ Nonsan-cheon (0.4). However, the endemicity in fish from ⑰ Geum-gang in Muju-gun was moderate (22.0). The endemicity of CsMc also showed moderate levels in fish from ㉒ Mangyeong-gang (26.5) and Tamjin-gang (㉓ Jangheung: 17.5 and ㉔ Gangjin: 26.1). In the water systems of Yeongsan-gang, the endemicity was low at 2.5 and 1.4 (㉕ Jiseok-cheon and ㉖ Yeongam-cheon). The endemicity was low in Seomjin-gang, i.e., ㉗ Osu-cheon in Imsil-gun (0.5), ㉘ Seomjin-gang in Sunchang-gun (3.9), ㉙ Songdae-cheon (2.3), and ㉚ Seomjin-gang in Gokseong-gun (3.2), except for that in ㉛ Gurye-gun (15.6). In the lower reaches of Seomjin-gang in Hadong-gun, Gyeongsangnam-do, the endemicity of CsMc was also as low as 0.8 (㉜ Hwagya-cheon), 0.02 (㉝ Akyang-cheon), 0.8 (㉞ Namsan-cheon), 0.004 (㉟ Heong-cheon), and 2.6 (㊱ Jugyo-cheon), respectively. The endemicity in the upper reaches of Nakdong-gang in Gyeongsangbuk-do was as high as 139.6 (㊲ Banbyun-cheon), 295.3 (㊳ Yongjeon-cheon), and 287.5 (㊴ Wi-cheon). However, the endemicity in the lower reaches of Nakdong-gang in Gyeongsangnam-do was not high at 48.1 (㊵ Yang-cheon), 8.3 (㊶ Deokcheon-gang), and 1.1 (㊷ Jisu-cheon and Haman-cheon). In streams of the East Coastal areas, the endemicity was not also high at 0.4 (㊸ Gigyae-cheon) and 5.6 (㊹ Taehwa-gang and Cheokgwa-cheon) (Table 3).

Collectively, the endemicity of CsMc was high in cyprinid fish from 3 (6.1%) regions in the upper reaches of Nakdong-



**Table 2.** Overall infection status of *Clonorchis sinensis* metacercariae (CsMc) in cyprinid fish by the water systems of Korea

Locality (Year) and fish sp.	No. of fish examined	No. of fish Infected	No. of CsMc detected	
			Range	Average
Hantan-/Imjin-gang and Han-gang				
①	425	38 (8.9)	1-11	2.2
⑦	100	40 (40.0)	1-1,012	35.6
⑧	411	180 (43.8)	1-654	37.8
⑪	129	7 (5.4)	1-3	1.6
Geum-gang				
⑰	37	36 (97.3)	1-141	90.4
⑱	235	64 (27.2)	1-924	27.0
⑳	28	12 (41.9)	1-81	19.3
㉑	16	7 (43.8)	1-3	1.7
Mangyeong-gang				
㉒-1	241	116 (48.1)	1-225	22.2
㉒-2	338	89 (21.8)	1-924	235.0
Tamjin-gang				
㉓-1	648	320 (49.4)	1-950	51.5
㉓-2	319	144 (45.1)	1-350	42.4
㉔	257	161 (62.7)	1-745	57.2
Yeongsan-gang				
㉕	48	33 (68.8)	1-3	6.6
㉖	19	8 (42.1)	1-20	6.8
Seomjin-gang				
㉗	148	33 (22.3)	1-24	4.4
㉘-1	108	69 (63.9)	1-79	11.1
㉘-2	488	215 (44.1)	1-484	
㉙	192	127 (66.1)	1-92	9.5
㉚-1	219	99 (45.2)	1-348	22.5
㉚-2	427	155 (36.3)	1-99	6.8
㉛	207	115 (55.6)	1-1,580	42.1
㉜	38	15 (39.5)	1-14	6.1
㉝	7	1 (14.3)	-	1.0
㉞	37	3 (8.1)	1-336	113.0
㉟	91	2 (2.2)	-	1.0
㊱	13	5 (38.5)	-	26.8
Nakdong-gang				
㊲	125	102 (81.6)	1-2,220	190.1
㊳	625	372 (59.5)	1-21,510	677.1
㊴-1	496	315 (63.5)	1-31,250	716.0
㊴-2	576	408 (70.8)	1-9,870	585.0
㊴-3	715	449 (62.8)	1-7,250	148.0
㊵-1	1,242	785 (63.2)	1-1,157	96.2
㊵-2	846	508 (60.0)	1-451	60.3
㊶	584	228 (39.0)	1-208	27.6
㊷	124	26 (21.0)	1-101	6.5
Streams in east coastal areas				
㊸	18	13 (72.2)	1-28	5.2
㊹	136	73 (53.7)	1-150	17.5

gang (Banbyeon-cheon, Yongjeon-cheon, and Wi-cheon). The moderate levels were observed in fishes from 8 (16.3%) survey regions, i.e., Munsan-cheon, Togyo-jeosuji, Geum-gang (Muju-gun), Soyang-cheon, Tamjin-gang (Jangheung-gun and Gangjin-

gun), Seomjin-gang (Gurye-gun), and Yang-cheon. The low endemicity was shown in fishes from 20 (40.8%) regions, which included Hantan-gang (Cheorwon-gun), Seom-gang (Wonju-si), Geum-gang (Geumsan-gun), Ji-cheon, Nonsan-cheon, Jis-

**Table 3.** Endemicity with *Clonorchis sinensis* metacercariae (CsMc) in cyprinid fish by the positive locality in Korea

Locality surveyed	Positive rate <sup>a</sup> of fish	Positive rate with CsMc in positive fish sp. <sup>b</sup>	Mean No. CsMc detected	Endemicity <sup>c</sup>
Hantan-/Imjin-gang and Han-gang				
①	26.9 (7/26)	8.9 (38/425)	2.2	0.05
⑦	87.5 (7/8)	40.0 (40/100)	35.6	12.5
⑧	73.3 (11/15)	43.8 (180/411)	37.8	12.1
⑪	15.0 (3/20)	5.4 (7/129)	1.6	0.01
Geum-gang				
⑰	25.0 (2/8)	97.3 (36/37)	90.4	22.0
⑱	58.8 (10/17)	58.8 (10/17)	27.0	4.3
⑳	50.0 (4/8)	50.0 (4/8)	19.3	4.0
㉑	50.0 (4/8)	50.0 (4/8)	1.7	0.4
Mangyeong-gang				
㉒	65.4 (17/26)	35.4 (205/579)	114.6	26.5
Tamjin-gang				
㉓	75.0 (24/32)	48.0 (464/967)	48.7	17.5
㉔	72.7 (8/11)	62.7 (161/257)	57.2	26.1
Yeongsan-gang				
㉕	55.6 (5/9)	68.8 (33/48)	6.6	2.5
㉖	50.0 (4/8)	42.1 (8/19)	6.8	1.4
Seomjin-gang				
㉗	54.5 (6/11)	22.3 (33/148)	4.4	0.5
㉘	54.2 (26/48)	47.7 (284/596)	14.9	3.9
㉙	36.8 (7/19)	66.1 (127/192)	9.5	2.3
㉚	63.3 (19/30)	39.3 (254/646)	12.9	3.2
㉛	66.7 (14/21)	55.6 (115/207)	42.1	15.6
㉜	33.3 (4/12)	39.5 (15/38)	6.1	0.8
㉝	14.3 (1/7)	14.3 (1/7)	1.0	0.02
㉞	8.3 (1/12)	8.1 (3/37)	113.0	0.8
㉟	18.2 (2/11)	2.2 (2/91)	1.0	0.004
㊱	25.0 (4/16)	38.5 (5/13)	26.8	2.6
Nakdong-gang				
㊲	90.0 (9/10)	81.6 (102/125)	190.1	139.6
㊳	73.3 (11/15)	59.5 (372/625)	677.1	295.3
㊴	96.8 (61/63)	65.6 (1,172/1,787)	452.8	287.5
㊵	94.6 (35/37)	61.9 (1,293/2,088)	82.1	48.1
㊶	76.9 (10/13)	39.0 (228/584)	27.6	8.3
㊷	81.8 (9/11)	21.0 (26/124)	6.5	1.1
Streams in east coastal areas				
㊸	11.1 (1/9)	72.2 (13/18)	5.2	0.4
㊹	60.0 (6/10)	53.7 (73/136)	17.5	5.6

<sup>a</sup>No. of positive fish species/No. of cyprinid fish spp. examined × 100.

<sup>b</sup>No. of fish infected/No. of fish examined in cyprinid fish spp. × 100.

<sup>c</sup>Endemicity:  $^a/100 \times ^b/100 \times$  mean No. of CsMc detected.

eok-cheon, Yeongam-cheon, Osu-cheon, Seomjin-gang (Sun-chang-gun), Songdae-cheon, Seomjin-gang (Gokseong-gun), Hwagya-cheon, Akyang-cheon, Namsan-cheon, Heong-cheon, Jugyo-cheon, Deokcheon-gang, Jisu-cheon and Haman-cheon, Gigyae-cheon, Taehwa-gang and Cheokgwacheon, respectively. No CsMc was detected in fish from 18 (36.7%) survey regions, 5 each in Hantan-/Imjin-gang, and streams in the East Coastal areas, 6 in Han-gang, and 2 in Ge-

um-gang (Table 4; Fig. 3).

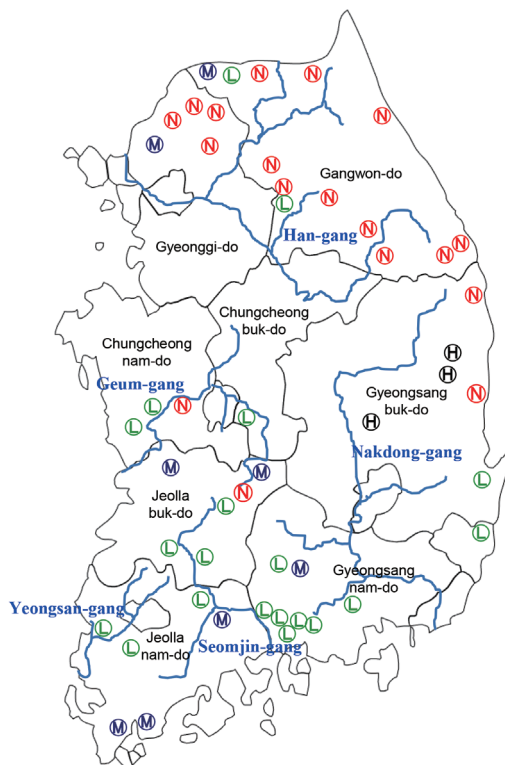
### SUSCEPTIBILITY OF CsMc IN THE INDEX FISH, *P. herzi*, BY SURVEYED AREAS

The striped shinner, *P. herzi*, (Fig. 4) is appropriate to be the index fish species to determine the endemicity of *C. sinensis* infection. This fish species is widespread in river water systems

**Table 4.** Distributions of endemicity with *C. sinensis* metacercariae in cyprinid fish by the water systems in Korea

Locality (River) surveyed	No. of localities by the endemicity <sup>a</sup>				
	Negative	Low	Moderate	High	Total (%)
Hantan-/Imjin-gang	5	1	2	0	8 (16.3)
Han-gang	6	1	0	0	7 (14.3)
Geum-gang	2	3	1	0	6 (12.2)
Mangyeong-gang	0	0	1	0	1 (2.0)
Yeongsan-gang	0	2	0	0	2 (4.1)
Tamjin-gang	0	0	2	0	2 (4.1)
Seomjin-gang	0	9	1	0	10 (20.4)
Nakdong-gang	0	2	1	3	6 (12.2)
Streams in east coast	5	2	0	0	7 (14.3)
Total (%)	18 (36.7)	20 (40.8)	8 (16.3)	3 (6.1)	49 (100)

<sup>a</sup>Positive rate of cyprinid fish spp./100×Positive rate with CsMc in positive cyprinid fish spp./100×Mean No. of CsMc detected (Negative: 0; Low: below 10; Moderate: 10.01-100; High: over 100.01).

**Fig. 3.** Distribution of the endemicity with CsMc (N: No CsMc; L: Low; M: Moderate; H: High endemic) in fish by the surveyed areas and water systems in Korea.

in Korea and is highly susceptible to CsMc. The SI of CsMc in index fish, *P. herzi*, from Hantan-gang ①, Munsan-cheon ⑦, and Seom-gang ⑪ was low at 0.5, 45.1, and 0.1, but that from Tokyo-jeosuji ⑧ was moderate at 76.3. In the water systems of Geum-gang, the SI showed moderate-level at 92.4, 98.1, and 50.7, in fish from Muju-gun ⑰, Geumsan-gun ⑱, and Ji-cheon ⑳. The SI in fish from Nonsan-cheon ㉑ was low at 1.0. The

**Fig. 4.** The index fish, *Puntungia herzi*, in the survey of *C. sinensis* metacercariae, which is very susceptible with CsMc and broadly lives in the water systems of Korea.

SI was high in fish from Mangyeong-gang (123.6) ㉒ and Tamjin-gang ㉔ (Gangjin-gun: 120.1). The SI of Tamjin-gang in Jangheung-gun ㉓ was moderate at 91.5. In the water systems of Yeongsan-gang, the SI was low at 8.7 and 5.8 in Jiseok-cheon ㉕ and Yeongam-cheon ㉖. The SI in Seomjin-gang was low at Imsil-gun (3.8) ㉗, Sunchang-gun (21.1) ㉘, Songdae-cheon (10.4) ㉙, and Gokseong-gun (32.7) ㉚ except for Gurye-gun (107.7) ㉛. In the lower reaches of Seomjin-gang in Hadong-gun, Gyeongsangnam-do, the SI was low at 2.8 in Hwagya-cheon ㉜, 9.2 in Namsan-cheon ㉝, and 12.1 in Jogyo-cheon ㉞. The SI in the upper reaches of Nakdong-gang in Gyeongsangbuk-do was very high at 615.0 in Banbyun-cheon ㉟, 1,060.7 in Yongjeon-cheon ㊱, and 1,046.5 in Wi-cheon ㊲. In the lower reaches of Nakdong-gang in Gyeongsangnam-do, the SI level was low in Deokcheon-gang (23.8) ㊴, Jisucheon and Haman-cheon (9.4) ㊵, but the SI was relatively high in Yang-cheon (148.3) ㊶. In streams in the east coastal areas of Korea, the SI was not high at 3.8 (㊸ Gigyae-cheon) and 22.2 (㊹ Taehwa-gang and Cheokgwa-cheon). Table 5 summarizes the SI levels along with surveyed areas.

Collectively, the SI in index fish, *P. herzi*, is fairly high in the

**Table 5.** Susceptibility index of *C. sinensis* metacercariae (CsMc) in the index fish, *Puntungia herzi*, by the surveyed locality in Korea

Locality surveyed	No. of fish examined	No. of fish infected	Mean No. CsMc detected	Susceptibility index <sup>a</sup>
Hantan-/Imjin-gang and Han-gang				
①	137	30 (21.9)	2.3	0.5
⑦ <sup>b</sup>	30	24 (85.3)	52.9	45.1
⑧ <sup>c</sup>	72	51 (70.8)	107.7	76.3
⑪	121	4 (3.3)	1.8	0.1
Geum-gang				
⑰	35	34 (97.1)	95.2	92.4
⑱	15	15 (100)	98.1	98.1
⑳	3	3 (100)	50.7	50.7
㉑ <sup>d</sup>	3	2 (66.7)	1.0	1.0
Mangyeong-gang				
㉒	185	155 (83.8)	147.5	123.6
Tamjin-gang				
㉓	209	206 (98.6)	92.8	91.5
㉔	70	70 (100)	120.8	120.8
Yeongsan-gang				
㉕ <sup>e</sup>	20	20 (100)	8.7	8.7
㉖ <sup>f</sup>	9	6 (66.7)	8.7	5.8
Seomjin-gang				
㉗ <sup>g</sup>	35	27 (77.1)	4.9	3.8
㉘	160	139 (86.9)	24.3	21.1
㉙	83	60 (72.3)	14.4	10.4
㉚	66	57 (86.4)	37.9	32.7
㉛	33	23 (69.7)	154.5	107.7
㉜	17	7 (41.2)	6.7	2.8
㉝	37	3 (8.1)	113.0	9.2
㉞ <sup>h</sup>	11	4 (36.4)	33.3	12.1
Nakdong-gang				
㉟	26	26 (100)	615.0	615.0
㊱	234	226 (96.6)	1,098.0	1,060.7
㊲	274	274 (100)	1,046.5	1,046.5
㊳	546	544 (99.6)	148.9	148.3
			148.3	
㊴	123	79 (64.2)	37.0	23.8
㊵ <sup>i</sup>	14	3 (21.4)	44.0	9.4
Streams in east coastal areas				
㊶	18	13 (72.2)	5.2	3.8
㊷	48	37 (77.1)	28.8	22.2

<sup>a</sup>Prevalence/100 × mean No. of CsMc detected.<sup>b</sup>*Pseudorasbora parva*.<sup>c</sup>16 *P. herzi*+56 *P. parva*.<sup>d</sup>2 *P. parva* + 1 *Sarcocheilichthys variegatus*.<sup>e</sup>1 *Squalidus gracilis majimae* + 19 *Squalidus chankaensis*.<sup>f</sup>2 *P. herzi* + 7 *Sarcocheilichthys nigripinnis*.<sup>g</sup>*S. chankaensis*.<sup>h</sup>4 *S. japonicus coreanus* + 1 *S. variegatus* + 6 *P. parva*.<sup>i</sup>1 *P. herzi* + 12 *S. gracilis majimae* + 1 *S. chankaensis*.

upper reaches of Nakdong-gang, such as Banbyun-cheon, Yongjeon-cheon, and Wi-cheon, where as it is relatively high in Soyang-cheon, Tamjin-gang (Gangjin-gun), Seomjin-gang (Gurye-gun), and Yang-cheon. The moderate levels of SI were observed in fishes from 5 (10.2%) surveyed regions, i.e., To-gyo-jeosuji, Geum-gang (Muju-gun and Geumsan-gun), Ji-

cheon, and Tamjin-gang (Jangheung-gun). The low SI was detected in *P. herzi* from 19 (38.8%) regions, i.e., Hantan-gang (Cheorwon-gun), Munsan-cheon, Seom-gang (Wonju-si), Nonsan-cheon, Jiseok-cheon, Yeongam-cheon, Osu-cheon, Seomjin-gang (Sunchang-gun and Gokseong-gun), Songdae-cheon, Hwagya-cheon, Akyang-cheon, Namsan-cheon,



**Table 6.** Distributions of susceptibility of *C. sinensis* metacercariae (CsMc) in index fish, *Puntungia herzi*, by the water systems in Korea

Locality (River) surveyed	No. of localities by the susceptibility <sup>a</sup> in index fish				Total (%)
	Negative	Low	Moderate	High	
Hantan-/Imjin-gang	5	2	1	0	8 (16.3)
Han-gang	6	1	0	0	7 (14.3)
Geum-gang	2	1	3	0	6 (12.2)
Mangyeong-gang	0	0	0	1	1 (2.0)
Yeongsan-gang	0	2	0	0	2 (4.1)
Tamjin-gang	0	0	1	1	2 (4.1)
Seomjin-gang	0	9	0	1	10 (20.4)
Nakdong-gang	0	2	0	4	6 (12.2)
Streams in east coast	5	2	0	0	7 (14.3)
Total (%)	18 (36.7)	19 (38.8)	5 (10.2)	7 (14.3)	49 (100)

<sup>a</sup>Prevalence/100 × mean No. of CsMc detected; Negative: No CsMc; Negative: 0; Low: below 10; Moderate: 10.01-100; High: over 100.01.

Heong-cheon, Jugyo-cheon, Deokcheon-gang, Jisu-cheon and Haman-cheon, Gigyae-cheon, Taehwa-gang, and Cheokgwa-cheon, respectively (Table 6).

### INFECTION TENDENCY OF CsMc BY THE SUBFAMILY GROUP IN CYPRINID FISH

The infection tendency of CsMc was investigated by the subfamily group in cyprinid fish from a highly endemic area, Wi-cheon (Gunwi-gun, Gyeongsangbuk-do). CsMc was detected in 545 (99.8%) out of 546 fishes in the gobioninid group-1, in which mean intensity was 934 PFI. The SI averaged 932.0. The SI was the highest in *S. gracilis majimae* (1,434.0). CsMc was detected in 49 (90.7%) out of 54 fishes in the gobioninid group-2 with mean intensity of 87.5 PFI. The SI was 79.4 in average. CsMc was detected in 361 (78.0%) out of 463 fishes in the acheilognathinid group. Their mean intensity was 39.2 PFI with an average SI of 30.6. CsMc was detected in 208 (32.2%) out of 646 fishes in the rasborinid group with the mean intensity of 12.6 PFI. The SI was 4.1 in average. The SI was the highest in *Z. platypus* at 6.8. Table 7 depicts the infection tendency of CsMc by the subfamily groups and species in cyprinid fish hosts from a highly endemic area, Wi-cheon, in Gunwi-gun, Gyeongsangbuk-do.

Our team also surveyed the infection status of CsMc in fish from a highly endemic Wi-cheon area for 10 years from 2011 to 2020 [25]. Since this area was regarded as the index site for CsMc infection, we examined the fish to gain insight on the CsMc infection status. The endemicity of CsMc in fish from Wi-cheon showed a tendency to decrease by chronological surveys [25]. Moreover, the infection status showed a certain

tendency in PFS according to the subfamily groups, i.e., Gobi-oninae, Acheilognathinae, and Rasborinae, in the Cyprinidae fish hosts of *C. sinensis*. This infection tendency of CsMc was also shown in fish from Yongjeon-cheon in Cheongsong-gun, Gyeongsangbuk-do and Yang-cheon in Sancheong-gun, Gyeongsangnam-do [26,29]. Although the number of fish species examined in 2 local sites was insufficient to compare clear relationship between subfamily groups of cyprinid fish, our findings suggested that the endemicity of CsMc is closely related to the fish groups in the cyprinid fishes. This host-parasite specific relationship may have originated from a long-lasting process of coevolution.

### INFECTION STATUS OF CsMc IN NON-CYPRINID FISH

We detected CsMc in 7 fish species of non-cyprinid, i.e., *Coreoperca herzi*, *Siniperca scherzeri*, *Odontobutis platycephala*, *Channa argus*, *Misgurnus anguillicaudatus*, *Micropterus salmoides*, and *Lepomis macrochirus* [28-31]. Infection status of CsMc by the fish species and by survey localities is summarized in Table 8. Out of these non-cyprinid fishes, 2 species, i.e., *C. herzi* and *S. scherzeri*, have been described as the second intermediate hosts of *C. sinensis*. Remaining 5 species are recently reported as the new second intermediate hosts [28-31]. Of these, some raw edible fishes, i.e., Mandarin fish (*S. scherzeri*), Korean aucha perch (*C. herzi*), Korean dark sleeper (*O. platycephala*), pond smelt (*Hypomesus nipponensis*), common carp (*Cyprinus carpio*), and crucian carp (*C. auratus*) are principally act as the infection source of human clonorchiasis in Korea. Fortunately, however, these fish species are less susceptible to CsMc [25,26,29].

**Table 7.** Infection tendency of *C. sinensis* metacercariae by the subfamily groups and species in cyprinid fish hosts from a highly endemic area, Wi-cheon, in Gunwi-gun, Gyeongsangbuk-do, Korea

Subfamily and species of cyprinid	No. of fish examined	No. (%) of fish infected	No. of CsMc detected		
			Range	Average	SI <sup>a</sup>
<b>Gobioninid group-1</b>					
<i>Pungtungia herzi</i>	274	274 (100)	3-31,250	1,047	1,047
<i>Squalidus japonicus coreanus</i>	117	117 (100)	2-8,460	775	775
<i>Squalidus gracilis majimae</i>	53	53 (100)	9-7,680	1,434	1,434
<i>Squalidus chankaensis</i>	46	46 (100)	14-7,250	549	549
<i>Sarcocheilichthys variegatus</i>	36	36 (100)	3-2,730	511	511
<i>Pseudorasbora parva</i>	16	15 (93.8)	1-2,225	725	680
<i>Sarcocheilichthys nigripinnis</i>	4	4 (100)	64-732	299	299
Subtotal	546	545 (99.8)	1-31,250	934	932
<b>Gobioninid group-2</b>					
<i>Pseudogobio esocinus</i>	26	24 (92.3)	1-504	138.0	127.4
<i>Microphysogobio koeensis</i>	10	9 (90.0)	1-139	21.7	19.5
<i>Hemibarbus longirostris</i>	9	7 (77.8)	2-10	5.1	4.0
<i>Abbottina springeri</i>	6	6 (100)	10-80	41.7	41.7
<i>Hemibarbus labeo</i>	1	1 (100)	-	9.0	9.0
<i>Microphysogobio jeoni</i>	1	1 (100)	-	365.0	365
<i>Ladislavia taczanowskii</i>	1	1 (100)	-	121.0	121
Subtotal	54	49 (90.7)	1-504	87.5	79.4
<b>Acheilognathinid group</b>					
<i>Acheilognathus koreensis</i>	197	181 (91.9)	1-678	29.4	27.0
<i>Acheilognathus yamatsutae</i>	118	60 (50.8)	1-136	11.8	6.0
<i>Acheilognathus rhombeus</i>	112	100 (89.3)	1-329	69.8	62.3
<i>Acheilognathus majusculus</i>	15	3 (20.0)	1-2	1.3	0.3
<i>Acheilognathus lanceolatus</i>	10	6 (60.0)	1-255	142.7	85.6
<i>Acanthorhodeus gracilis</i>	6	6 (100)	2-15	6.8	6.8
<i>Acanthorhodeus macropterus</i>	4	4 (100)	2-87	42.5	42.5
<i>Rhodeus pseudosericeus</i>	1	1 (100)	-	72.0	72.0
Subtotal	463	361 (78.0)	1-678	39.2	30.6
<b>Rasborinid group</b>					
<i>Zacco platypus</i>	382	195 (51.0)	1-420	13.3	6.8
<i>Zacco temminckii</i>	134	6 (4.5)	1-4	1.7	0.08
<i>Zacco koreanus</i>	120	4 (3.3)	1-3	1.5	0.05
<i>Opsariichthys uncirostris</i>	10	3 (30.0)	-	1.0	0.3
Subtotal	646	208 (32.2)	1-420	12.6	4.1

<sup>a</sup>Prevalence/100 × mean No. of CsMc detected.**Table 8.** Infection status of *C. sinensis* metacercariae in non-cyprinid fish from the water systems of Korea

Fish species	Locality (No. of fish positive/No. of fish examined; Mean No. of CsMc detected) surveyed
<i>Coreoperca herzi</i>	Soyang-cheon (1/23; 1.0), Tamjin-gang (1/3; 1.0), Yongjeon-cheon (2/66; 1.0), Wi-cheon (3/14; 1.7), Yang-cheon (1/30; 1.0), Deokcheon-gang (5/58; 1.6)
<i>Siniperca scherzeri</i>	Ji-cheon (2/11; 1.5), Geum-gang in Muju (1/1; 2.0), Yongjeon-cheon (2/3; 2.0), Wi-cheon (1/1; 6.0)
<i>Odontobutis platycephala</i>	Soyang-cheon (1/57; 1.0), Tamjin-gang (1/12; 1.0), Yongjeon-cheon (2/41; 2.0), Wi-cheon (4/15; 2.0), Cheokgwa-cheon (1/6; 1.0)
<i>Channa argus</i>	Yang-cheon (1/6; 1.0)
<i>Misgurnus anguillicaudatus</i>	Wi-cheon (2/6; 1.0)
<i>Micropterus salmoides</i>	Wi-cheon (2/10; 3.0)
<i>Lepomis macrochirus</i>	Yang-cheon (2/50; 1.0)

**Table 9.** The fish intermediate hosts of *C. sinensis* in Korea

Family	Genus	Species <sup>a</sup>
Cyprinidae	<i>Abbottina</i>	<i>A. rivularis</i> , <i>A. springeri</i>
	<i>Acanthorhodeus</i>	<i>A. gracilis</i> , <i>A. macropterus</i> <sup>b</sup>
	<i>Acheilognathus</i>	<i>A. asmussi</i> , <i>A. lanceolata</i> , <i>A. signifer</i> , <i>A. yamatsutae</i>
	<i>Aphyocypris</i>	<i>A. chinensis</i>
	<i>Carassius</i>	<i>C. auratus</i>
	<i>Chanodichthys</i>	<i>C. erythropterus</i>
	<i>Coreoleuciscus</i>	<i>C. splendidus</i>
	<i>Cyprinus</i>	<i>C. carpio</i>
	<i>Gnathopogon</i>	<i>G. strigatus</i> , <i>G. astromaculatus</i> , <i>G. coreanus</i>
	<i>Hemiculter</i>	<i>H. leucisculus</i>
	<i>Hemibarbus</i>	<i>H. labeo</i> , <i>H. longirostris</i> , <i>H. mylodon</i> <sup>b</sup>
	<i>Ladislabia</i>	<i>L. taczanowski</i> <sup>b</sup>
	<i>Microphysogobio</i>	<i>M. koreensis</i> , <i>M. yaluensis</i> , <i>M. longidorsalis</i> <sup>b</sup> , <i>M. jeoni</i> <sup>b</sup>
	<i>Opsariichthys</i>	<i>O. uncirostris amurensis</i>
	<i>Pseudogobio</i>	<i>P. esocinus</i>
	<i>Pseudorasbora</i>	<i>P. parva</i>
	<i>Puntungia</i>	<i>P. herzi</i>
	<i>Rhodeus</i>	<i>R. ocellatus</i> , <i>R. pseudosericeus</i> <sup>b</sup>
	<i>Rhynchocypris</i>	<i>R. oxycephalus</i> <sup>b</sup>
	<i>Saurogobio</i>	<i>S. dabryi</i>
	<i>Sarcocheilichthys</i>	<i>S. nigripinnis morii</i> , <i>S. variegatus wakiyae</i>
<i>Squaliobarbus</i>	<i>S. curriculum</i>	
<i>Squalidus</i>	<i>S. japonicus coreanus</i> , <i>S. gracilis majimae</i> , <i>S. chankaensis tsuchigae</i> <sup>b</sup> , <i>S. multimaculatus</i> <sup>b</sup>	
<i>Tribolodon</i>	<i>T. hakonensis</i>	
<i>Zacco</i>	<i>Z. platypus</i> , <i>Z. temminckii</i> , <i>Z. koreanus</i> <sup>b</sup>	
Bagridae	<i>Coreobagrus</i>	<i>C. brevicorpus</i>
Pristigasteridae	<i>Ilisha</i>	<i>I. elongata</i>
Belontiidae	<i>Macropodus</i>	<i>M. ocellatus</i>
Centropomidae	<i>Coreoperca</i>	<i>C. herzi</i>
	<i>Siniperca</i>	<i>S. scherzei</i>
Odontobutidae	<i>Odontobutis</i>	<i>O. platycephala</i> <sup>b</sup>
Osmeridae	<i>Hypomesus</i>	<i>H. olidus</i>
Cobitidae	<i>Misgurnus</i>	<i>M. anguillicaudatus</i> <sup>b</sup>
Channidae	<i>Channa</i>	<i>C. argus</i> <sup>b</sup>
Centrarchidae	<i>Lepomis</i>	<i>L. macrochirus</i> <sup>b</sup>
	<i>Micropterus</i>	<i>M. salmoides</i> <sup>b</sup>

<sup>a</sup>A total of 58 fish species (10 families) were listed as the second intermediate hosts of *C. sinensis* in Korea. Among them, 41 species were collectively listed in Rim [33] and Sohn [39], and <sup>b</sup>remain 17 species were newly added by our recent reports [22,26,29-32].

### FISH INTERMEDIATE HOSTS OF *C. sinensis* IN KOREA

In 1917, Kobayashi [8] first described CsMc in Korea, which were detected from 3 fish species, i.e., *A. rivularis*, *C. auratus* and *P. parva*. Thereafter, many workers have reported the fish intermediate hosts of *C. sinensis* in Korea [9-31]. A total of 36 fish species in 3 families (Cyprinidae: 34 spp.; Bagridae: 1 sp.; and Clupeidae: 1 sp.) has been recorded as the second intermediate hosts of *C. sinensis* in Korea [32]. Sohn [38] rearranged the fish intermediate hosts of *C. sinensis*, and nominated a total of 40 fish species in 6 families (Cyprinidae: 34 spp.;

Bagridae: 1 sp.; Pristigasteridae: 1 sp.; Osphronemidae: 1 sp.; Percichthyidae: 2 spp.; and Osmeridae: 1 sp.). We recently added 17 new fish species to the list of the second intermediate hosts of *C. sinensis* in Korea, which included *Squalidus chankaensis*, *S. multimaculatus*, *Hemibarbus mylodon*, *Microphysogobio jeoni*, *M. longidorsalis*, *Ladislabia taczanowskii*, *Acheilognathus koreensis*, *A. majusculus*, *Acanthorhodeus macropterus*, *Rhodeus pseudosericeus*, *Zacco koreanus*, *Rhynchocypris oxycephalus*, *Odontobutis platycephala*, *Channa argus*, *Misgurnus anguillicaudatus*, *Micropterus salmoides*, and *Lepomis macrochirus* [21,25,28-31]. Among them, 2 fish species, i.e., largemouth bass (*M. salmoides*) and blue gill (*L. macrochirus*), are exotic species. Table 9

summarizes a total of 58 fish species in 10 families, which are currently designated as the second intermediate hosts of *C. sinensis* in Korea.

### CONCLUDING REMARK

Clonorchiasis is one of the major fish-borne trematodiasis and is still endemic in Korea. This endemic disease causes a significant public health problem among residents in major river basins in Korea. The infection status of CsMc in freshwater fish hosts is directly associated with transmission of human clonorchiasis. This article reviewed data mostly obtained from our surveys from 2010 to 2020. The status of CsMc infection was analyzed on a total of 17,792 cyprinid fish of 49 species among all fishes collected from 9 main river systems in Korea, such as Hantan-/Imjin-gang, Han-gang, Geum-gang, Mangyeong-gang, Yeongsan-gang, Tamjin-gang, Seomjin-gang, Nakdong-gang, and streams in the east coastal areas. The high endemicity was observed in the cyprinid fish from upper reaches of Nakdong-gang in Gyeongsangbuk-do. CsMc infections were closely related to subfamily groups in the cyprinid fish hosts in a highly endemic area. The infection status of CsMc in index fish, *P. herzi*, might represent the overall infection patterns of the fish hosts. A total 58 fish species in 10 families have been designated as the second intermediate hosts of *C. sinensis*. In Korea, the endemicity of CsMc infections in fish hosts and the incidence of human clonorchiasis are gradually decreasing. However, continuous monitoring of infection status of fish hosts may be required to control and management of clonorchiasis affecting humans and reservoir hosts in this country.

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### CONFLICT OF INTEREST

The author has no conflicts of interest concerning the work reported in this paper.

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