Infection Status with *Clinostomum complanatum* Metacercariae in Fish from Water Systems of Nakdong-gang (River) in Korea

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**Abstract:** The infection status of *Clinostomum complanatum* metacercariae (CcMc) was broadly surveyed in fishes from water systems of Nakdong-gang (River) in the Republic of Korea (Korea) for 5 years. All 4,468 fishes collected were individually examined by the artificial digestion methods. No CcMc were detected in fishes from Naeseongcheon in Yecheon-gun, Gigyecheon in Gyeongju-si, Gyeongsangbuk-do, and Hamancheon in Haman-gun, Gyeongsangnam-do. In fishes from Wicheon in Gunwi-gun, Gyeongsangbuk-do, CcMc were detected in 180 (15.4%) out of 1,168 fishes and their intensity was 5.8 per fish infected. The prevalences with CcMc in positive fish species from 3 other surveyed areas, i.e., Banbyeoncheon in Yeonggang-gun, Nakdong-gang in Sangju-si and Hoecheon in Goryeong-gun, in Gyeongsangbuk-do were 19.3%, 33.3%, and 19.0% and their intensities were 1.5, 17.6, and 2.6 per fish infected respectively. In fishes from Yangcheon in Sancheong-gun, Gyeongsangnam-do, CcMc were detected in 811 (45.6%) out of 1,779 fishes examined and their intensity was 9.8 per fish infected. The prevalence was most high in *Squalidus* spp. (87.7%) and followed by acheilognathinid fish (66.8%), *Pungtungia herzi* (52.0%), rasborinid fish (39.6%) and *Hemibarbus* spp. (25.3%) from Yangcheon. The intensity was also most high in *Squalidus* spp. (27.0). The endemicity with CcMc was very low in fishes from water systems of Nakdong-gang in the east coast of Korea. Conclusively, it is confirmed that various fish species act as the second intermediate hosts of *C. complanatum*, and the endemicities of CcMc are more or less different by the fish species and group from Yangcheon, in Sacheong-gun, Gyeongsangnam-do, Korea

**Key words:** Clinostomum complanatum, metacercaria, Nakdong-gang, Yangcheon

**INTRODUCTION**

*Clinostomum complanatum* (Digenea: Clinostomidae) is a laryngeal fluke in avian and mammalian hosts. This species of trematode is sometimes to be infected in humans through the consumption of raw fish meat. More than 25 human cases were mainly reported in Japan and the Republic of Korea [1-8]. In Korea, total 6 cases were reported since the first occurrence in 1994 [3-8].

*Clinostomum* spp. laryngeal flukes commonly have 2 intermediate hosts in the life cycle. The freshwater snails in the family Lymnaeidae, i.e., *Lymnaea* spp. and *Radix* spp., serve as the first intermediate hosts, and freshwater and brackish water fish act as the second intermediate hosts. In Korea, Chung et al. [9] recorded 12 species of freshwater fishes as the second intermediate hosts of *C. complanatum*, and Chung et al. [10] also described the cercariae and redia of *C. complanatum*, which were detected in *Radix auricularia coreana* from a pond, an enzootic focus of this trematode, in Uiseong-gun (gun = county), Gyeongsangbuk-do (do = Province). Rim et al. [11] detected *C. complanatum* metacercariae (CcMc) in 2 fish species, *Pseudorasbora parva* and *Squalidus chanbaensis tsuchigae*, from the streams of Taewha-gang (gang means river) and Hyeongsangang located in the southeast regions of Korea. Recently, Sohn et al. [12] found CcMc in 2 fish species, *Punctungia herzi* and *Hemibarbus longirostris*, from Chatancheon (a stream of Hantan-gang) in Yecheon-gun, Gyeongsang-do.

On the other hand, the infection status of zoonotic trematode metacercariae (ZTM) in the second intermediate hosts are used as the important epidemiological indices. Thus, many Korean workers have investigated the infection status with ZTM in fishes from various endemic areas to estimate the en-
demicities of specific zoonotic trematode, i.e., Clonorchis sinensis, Metagonimus spp. including M. yokogawai, Centrocestus armatus and Isthmiophora hortensis, infections [13-19]. However, the infection status of CcMc was rarely surveyed and has not been broadly investigated in freshwater fish from the water systems of Korea. Therefore, in the present study, we are going to investigate the infection status of CcMc in freshwater fish from the water systems of Nakdong-gang in Korea.

MATERIALS AND METHODS

Fish collection (Fig. 1)

Water systems of Nakdong-gang in Gyeongsangbuk-do: Fishes from Naeseongcheon (① in Fig. 1) in Yecheon-gun (2014: 103 fishes in 15 spp.), Banbyeoncheon (②) in Yeongyang-gun (2015: 161 fishes in 12 spp.), Wicheon (③) in Gunwi-gun (2013: 107 fishes in 12 spp.; 2014: 338 fishes in 24 spp.; 2015: 245 fishes in 19 spp.; 2016: 279 fishes in 24 spp.; 2017: 199 fishes in 17 spp.) Nakdong-gang (④) in Sangju-si (si = city), (2017: 143 fishes in 14 spp.) and Hoecheon (⑤) in Goryeong-gun (2013: 165 fishes in 13 spp.) were examined.

Water systems of Nakdong-gang in Gyeongsangnam-do: Fishes from Yangcheon (⑥) in Sancheong-gun (2013: 644 fishes in 20 spp.; 2014: 291 fishes in 14 spp.; 2015: 183 fishes in 15 spp.; 2016: 253 fishes in 16 spp.; 2017: 408 fishes in 22 spp.), Jisucheon (⑦) in Jinju-si (2014: 94 fishes in 11 spp.)

Fig. 1. Surveyed areas in water systems of Nakdong-gang (River) in Korea: ① Naeseongcheon in Yecheon-gun, ② Banbyeoncheon in Yeongyang-gun, ③ Wicheon in Gunwi-gun, ④ Nakdong-gang in Sangju-si, ⑤ Hoecheon in Goryeong-gun, Gyeongsangbuk-do, ⑥ Yangcheon in Sancheong-gun, ⑦ Jisucheon in Jinju-si, ⑧ Hamancheon in Haman-gun, Gyeongsangnam-do, ⑨ Wangpicheon in Uljin-gun, ⑩ Osipcheon in Yeongdeok-gun, ⑪ Gigyecheon in Gyeongju-si, Gyeongsangbuk-do, ⑫ Cheokgwacheon, and ⑬ Taehwagang in Ulsan-si (Metropolitan City).
and Hamancheon (8) in Haman-gun (2014: 73 fishes in 9 spp.) were examined.

Water systems of Nakdong-gang in the east coast of Korea: Fishes from Wangpicheon (9) in Uljin-gun (2015: 239 fishes in 13 spp.), Osipcheon (10) in Yeongdeok-gun (2015: 122 fishes in 11 spp.) and Gigyecheon (11) in Gyeongju-si (2015: 111 fishes in 11 spp.), Gyeongsangbuk-do, Cheokgwacheon (12) (2015: 221 fishes in 10 spp.) and Taehwagang (13) (2015: 89 fishes in 14 spp.) in Ulsan Metropolitan City were examined.

Examination methods

All collected fishes with ice were transferred to the laboratory of the Department of Parasitology and Tropical Medicine, Gyeongsang National University College of Medicine, Jinju, Korea. After the identification of fish species, they were individually ground with a mortar. Each ground fish meat was mixed with artificial gastric juice and the mixture was incubated at 36°C for 2 hr. The digested material of each fish was washed with 0.85% saline until the supernatant is cleared and examined with naked eyes and under a stereomicroscope. The excysted CcMc (Fig. 2) were separately collected [20] and were counted to get hold of infection rates (%) and densities (No. of CcMc per fish infected) by fish species.

RESULTS

Infection status with CcMc in fish from Nakdong-gang in Gyeongsangbuk-do

No CcMc were detected in fishes from Naeseongcheon in Yecheon-gun. In fishes from Wicheon in Gunwi-gun, CcMc were detected in 180 (15.4%) out of 1,168 fishes and their intensity was 5.8 per fish infected. The prevalences by the year examined were 15.0-27.0% (20.5% in average) in the positive fish species from Wicheon. The infection status with CcMc by the fish species and examined year in Wicheon was detailedly shown in Table 1. In positive fish species from another surveyed areas, i.e., Banbyeoncheon in Yeongyang-gun, Nakdong-gang in Sangju-si and Hoecheon in Goryeong-gun, the prevalence with CcMc was 23.8% in average and their intensity was 9.2 per fish infected. The infection status with CcMc by the fish species and survey areas was detailedly revealed in Table 2.

Infection status with CcMc in fish from Nakdong-gang in Gyeongsangnam-do

No CcMc were detected in fishes from Hamancheon in Haman-gun. In fishes from Yangcheon in Sangju-si and Hoecheon in Goryeong-gun, CcMc were detected in 811 (45.6%) out of 1,779 fishes examined and their intensity was 9.8 per fish infected. The prevalences by the year examined were 38.6-62.8% (51.4% in average) in the positive fish species from Yangcheon. The infection status with CcMc by the fish species and examined year in Yangcheon was detailedly designated in Table 3. Only one CcMc was found in a S. gracilis majimae from Jisucheon in Jinju-si. The infection tendency with CcMc by the fish groups was somewhat revealed in fishes from Yangcheon. 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 was also most high in Squalidus spp. (27.0) and followed by acheilognathinid fish (8.9), P. herzi (6.6), rasborinid fish (3.7) and Hemibarbus spp. (2.5) (Table 4).
No CcMc were detected in fishes from Gigecheon (a branch of Hyeongsangang) in Gyeongju-si, Gyeongsangbuk-do. In fishes from water systems of Nakdong-gang in the east coast of Korea, the endemicity with CcMc was very low. The infection status with CcMc by the survey areas and fish species examined was detailedly revealed in Table 5.

### Table 1. Infection status of Clinostomum complanatum metacercariae by the fish species and examined year in Wicheon in Gunwi-gun, Gyeongsangbuk-do, Korea

| Year and fish examined | No. of fish examined | No. of fish infected (%) | No. of CcMc detected |
|------------------------|----------------------|--------------------------|----------------------|
|                        | Range                | Average                  |
| 2013                   |                      |                          |
| Pungtungia herzi       | 20                   | 1 (5.0)                  | -                    | 2.0 |
| Acheilognathus koreensis | 10                   | 4 (40.0)                 | 1-7                  | 3.5 |
| Squalidus gracilis majimae | 9                   | 3 (33.3)                 | -                    | 1.0 |
| Subtotal               | 39                   | 8 (20.5)                 | 1-7                  | 2.4 |
| 2014                   |                      |                          |
| Zacco platypus         | 60                   | 4 (6.7)                  | 1-7                  | 3.0 |
| Zacco temminckii       | 50                   | 2 (4.0)                  | 1-3                  | 2.0 |
| Pungtungia herzi       | 47                   | 16 (34.0)                | 1-34                 | 7.1 |
| Acheilognathus koreensis | 29                   | 13 (44.8)                | 1-32                 | 6.1 |
| Acheilognathus yamatsutae | 25                   | 1 (4.0)                  | -                    | 1.0 |
| Squalidus japonicus coreanus | 14                  | 11 (78.6)                | 1-48                 | 7.9 |
| Carassius auratus      | 13                   | 1 (7.7)                  | -                    | 2.0 |
| Squalidus gracilis majimae | 13                  | 2 (15.4)                 | -                    | 1.0 |
| Subtotal               | 251                  | 50 (19.9)                | 1-48                 | 6.0 |
| 2015                   |                      |                          |
| Zacco platypus         | 50                   | 2 (4.0)                  | 2-7                  | 4.5 |
| Zacco temminckii       | 49                   | 1 (2.0)                  | -                    | 1.0 |
| Pungtungia herzi       | 41                   | 8 (19.5)                 | 1-11                 | 3.6 |
| Acheilognathus koreensis | 29                   | 20 (69.0)                | 1-20                 | 4.3 |
| Acheilognathus yamatsutae | 16                   | 1 (6.3)                  | -                    | 1.0 |
| Squalidus japonicus coreanus | 15                  | 4 (26.7)                 | 8-47                 | 21.0 |
| Squalidus gracilis majimae | 11                  | 4 (36.4)                 | 1-16                 | 5.8 |
| Abbottina springeri    | 4                    | 1 (25.0)                 | -                    | 3.0 |
| Subtotal               | 215                  | 50 (19.9)                | 1-47                 | 5.7 |
| 2016                   |                      |                          |
| Zacco platypus         | 63                   | 1 (1.6)                  | -                    | 5.0 |
| Acheilognathus koreensis | 55                   | 31 (66.4)                | 1-27                 | 5.1 |
| Pungtungia herzi       | 38                   | 11 (28.9)                | 1-8                  | 2.8 |
| Acheilognathus yamatsutae | 29                   | 2 (6.9)                  | 2-4                  | 3.0 |
| Acheilognathus rhombeus | 15                   | 7 (46.7)                 | 1-3                  | 2.1 |
| Squalidus japonicus coreanus | 9                   | 3 (33.3)                 | 8-34                 | 19.0 |
| Squalidus gracilis majimae | 2                   | 2 (100)                  | -                    | 32.0 |
| Subtotal               | 211                  | 57 (27.0)                | 1-59                 | 5.9 |
| 2017                   |                      |                          |
| Zacco platypus         | 50                   | 3 (6.0)                  | 2-3                  | 2.3 |
| Squalidus japonicus coreanus | 31                  | 7 (22.6)                 | 1-33                 | 8.6 |
| Acheilognathus yamatsutae | 27                   | 2 (7.4)                  | -                    | 1.0 |
| Pungtungia herzi       | 26                   | 5 (19.2)                 | 1-13                 | 4.6 |
| Acheilognathus koreensis | 26                   | 7 (26.9)                 | 1-42                 | 8.6 |
| Subtotal               | 160                  | 24 (15.0)                | 1-42                 | 6.3 |
| Total                  | 876                  | 180 (20.5)               | 1-59                 | 5.8 |

**Discussion**

In the present study, CcMc were found in total 23 species of fish from the streams in the east coast of Korea. The infection status with CcMc by the survey areas and fish species examined was detailedly revealed in Table 5.
freshwater fishes from the water systems of Nakdong-gang in Korea. Among these fish hosts, 10 ones, *A. koreensis*, *A. rhombeus*, *A. yamatsutae*, *C. auratus*, *H. longirostris*, *P. parva*, *P. herzi*, *S. chankae tsuchigae*, *S. gracilis maejimae*, and *Z. temminkii*, have been previously reported in Korea [9,11,12]. Therefore, a total of 26 fish species including 3 ones, *Cobitis sinensis*, *Microphugogobio yaluensis* and *Rhodeus uyekii*, formerly reported in Chung et al. [9] are to be listed as the second intermediate hosts of *C. complanatum* in Korea. In Japan, less than 8 fish species, i.e., *Carassius* spp. including *C. auratus*, *Cyprinus carpio*, *P. parva*, *Pseudogobio esocinus*, *Rhodeus ocellatus*, and *R. lanceolatus* (syn. *Acheilognathus lanceolatus*), were reported as the second intermediate hosts of *C. complanatum* until 1992 [21].

The prevalence of CcMc was most high in the positive fish group from Yangcheon (51.4%) in Sancheong-gun, Gyeongsangnam-do, followed by that from Nakdong-gang in Sangju-si, and those from remain other areas were relatively low (7.1-20.5%). Even no CcMc were detected in fishes from 3 survey areas, Naeseongcheon in Yecheon-gun, Gigyecheon in Gyeongju-si, Gyeongsangbuk-do and Hamancheon in Haman-gun, Gyeongsangnam-do. The intensity of infection with CcMc was most high in the positive fish group from Nakdong-gang (17.6 per fish infected) in Sangju-si, followed by that from Yangcheon (9.8) in Sancheong-gun, and those from remain other areas were relatively low (1.0-5.8). The reason why the more higher intensity in fish from Nakdong-gang in Sangju-si is 2 crucian carps, *C. auratus*, heavily infected (86 and 147 CcMc). Above findings suggested that the endemicities of CcMc are relatively high in fish from Yangcheon in Sancheong-gun and Nakdong-gang in Sangju-si, and relatively low in fish from other areas like in previous studies in Korea [9,11,12].

In the highly endemic area of CcMc, Yangcheon in Sancheong-gun, Gyeongsangnam-do, the endemicity was revealed a certain tendency by the subfamily groups in cyprinid fish. The endemicity (average CcMc intensity × prevalence/100) was 8.47 in gobioninid (Gobioninae) fish, i.e., *Squalidus* spp., 5.95 in acheilognathinid (Acheilognathininae) fish, 1.47 in rasborinid (Rasborininae) fish group, *Zacco* spp. The endemicity in the gobioninid fish was most high in *Squalidus* spp., 26.38, followed by in *P. herzi*, 3.43, and in *Hemibarbus* spp., 2.6. From the above findings, we could know that the susceptibility of *C. complanatum* cercariae is relatively high in *Squalidus* spp. and the endemicities of CcMc are more or less different by the fish group and species from Yang-

| Locality and fish sp. | No. of fish examined | No. of fish infected (%) | No. of CcMc detected |
|----------------------|----------------------|--------------------------|----------------------|
|                      |                      |                          | Range               | Average             |
| Banbyeoncheon in Yeongyang-gun |                      |                          |                      |                    |
| *Pungtungia herzi*   | 26                   | 2 (7.7)                  | 1-2                 | 1.5                |
| *Acheilognathus koreensis* | 18                 | 7 (38.9)                 | 1-5                 | 1.6                |
| *Squalidus gracilis maejimae* | 8                  | 1 (12.5)                 | -                   | 1.0                |
| *Abottinia springeri* | 5                    | 1 (20.0)                 | -                   | 1.0                |
| Subtotal             | 57                   | 11 (19.3)                | 1-5                 | 1.5                |
| Nakdonggang in Sangju-si |                     |                          |                      |                    |
| *Acheilognathus lanceolatus* | 31                 | 9 (29.0)                 | 1-11                | 4.0                |
| *Carassius auratus*  | 9                    | 2 (22.2)                 | 86-147              | 116.5              |
| *Acheilognathus rhombeus* | 9                  | 5 (55.6)                 | 3-26                | 11.6               |
| *Hemibarbus labeo*   | 6                    | 1 (16.7)                 | -                   | 4.0                |
| *Hemibarbus longirostris* | 2                 | 2 (100)                  | 1-2                 | 1.5                |
| Subtotal             | 57                   | 19 (33.3)                | 1-147               | 17.6               |
| Hoecheon in Goryeong-gun |                     |                          |                      |                    |
| *Hemiculter eigenmanni* | 34                 | 3 (8.8)                  | 1-3                 | 1.7                |
| *Pseudorasbora parva* | 16                  | 5 (31.3)                 | 1-10                | 3.2                |
| *Hemibarbus labeo*   | 7                    | 2 (28.6)                 | -                   | 3.0                |
| *Squalidus japonicus coreanus* | 1                | 1 (100)                  | -                   | 1.0                |
| Subtotal             | 58                   | 11 (19.0)                | 1-10                | 2.6                |
| Total                | 172                  | 41 (23.8)                | 1-147               | 9.2                |
Table 3. Infection status of Clinostomum complanatum metacercariae by the fish species and examined year in Yangcheon in Sanchezong-gun, Gyeongangnam-do, Korea

| Year and fish examined | No. of fish examined | No. of fish infected (%) | No. of CcMc detected |
|------------------------|----------------------|--------------------------|----------------------|
|                        |                      |                          | Range               | Average   |
| **2013**               |                      |                          |                      |           |
| Pungtungia herzi       | 142                  | 86 (60.6)                | 1-34                 | 5.9       |
| Zacco koreanus         | 94                   | 49 (52.1)                | 1-22                 | 3.3       |
| Acheilognathus majaclusus | 85                 | 66 (77.6)                | 1-22                 | 6.8       |
| Zacco platypus         | 55                   | 35 (63.6)                | 1-8                  | 2.9       |
| Squalidus chaikaensis  | 51                   | 51 (100)                 | 1-143                | 32.6      |
| Hemibarbus longirostris | 34                 | 7 (20.6)                 | 1-5                  | 1.9       |
| Acheilognathus koreensis | 31                | 28 (90.3)                | 1-14                 | 6.5       |
| Squalidus japonicus coreanus | 30             | 30 (100)                 | 2-46                 | 19.5      |
| Carassius auratus      | 20                   | 5 (25.0)                 | 1-5                  | 2.4       |
| Acanthorhodeus gracilis | 20                 | 5 (25.0)                 | 1-6                  | 3.2       |
| Acanthorhodeus macropterus | 17             | 1 (5.9)                  | -                    | 1.0       |
| Acheilognathus rhombeus | 8                   | 5 (62.5)                 | 1-7                  | 2.2       |
| Acheilognathus yamatsutae | 1                 | 1 (100)                  | -                    | 5.0       |
| Subtotal               | 588                  | 369 (62.8)               | 1-143                | 10.0      |
| **2014**               |                      |                          |                      |           |
| Pungtungia herzi       | 65                   | 31 (47.7)                | 1-14                 | 63.1      |
| Acheilognathus majaclusus | 40                 | 32 (80.0)                | 1-18                 | 3.5       |
| Zacco koreanus         | 40                   | 15 (37.5)                | 1-6                  | 1.9       |
| Coreperca herzi        | 30                   | 2 (6.7)                  | -                    | 1.0       |
| Zacco platypus         | 25                   | 10 (40.0)                | 1-6                  | 2.8       |
| Squalidus japonicus coreanus | 25             | 25 (100)                 | 7-67                 | 23.2      |
| Acheilognathus koreensis | 13                | 10 (76.9)                | 1-17                 | 7.4       |
| Odontobutis platycephala | 13              | 2 (15.4)                 | 5-10                 | 7.5       |
| Acheilognathus yamatsutae | 10                | 7 (70.0)                 | 1-6                  | 2.9       |
| Hemibarbus longirostris | 7                   | 2 (28.6)                 | -                    | 1.0       |
| Subtotal               | 268                  | 126 (47.0)               | 1-67                 | 7.3       |
| **2015**               |                      |                          |                      |           |
| Pungtungia herzi       | 40                   | 19 (47.5)                | 1-45                 | 9.9       |
| Zacco temminckii       | 40                   | 8 (20.3)                 | 1-12                 | 4.1       |
| Zacco platypus         | 40                   | 20 (50.0)                | 1-22                 | 5.2       |
| Hemibarbus longirostris | 25                  | 6 (24.0)                 | 1-4                  | 3.3       |
| Acheilognathus majaclusus | 9                   | 2 (22.2)                 | 14-40                | 27.0      |
| Acheilognathus koreensis | 7                   | 7 (100)                  | 3-74                 | 16.1      |
| Acheilognathus yamatsutae | 3                  | 1 (33.3)                 | -                    | 1.0       |
| Squalidus japonicus coreanus | 2             | 1 (50.0)                 | -                    | 12.0      |
| Subtotal               | 166                  | 64 (38.6)                | 1-74                 | 8.2       |
| **2016**               |                      |                          |                      |           |
| Acheilognathus koreensis | 40                 | 34 (85.0)                | 1-25                 | 6.2       |
| Pungtungia herzi       | 33                   | 13 (39.4)                | 1-8                  | 2.8       |
| Zacco plagipus         | 32                   | 9 (28.1)                 | 1-10                 | 3.2       |
| Acheilognathus majaclusus | 23                | 15 (65.2)                | 1-16                 | 4.1       |
| Carassius auratus      | 22                   | 1 (4.5)                  | -                    | 1.0       |
| Zacco temminckii       | 21                   | 2 (9.5)                  | 1-3                  | 2.0       |
| Zacco koreanus         | 20                   | 11 (55.0)                | 1-8                  | 3.7       |
| Acheilognathus rhombeus | 20                  | 16 (76.0)                | 1-13                 | 6.1       |
| Acheilognathus yamatsutae | 13                | 9 (69.2)                 | 1-14                 | 4.6       |
| Squalidus japonicus coreanus | 5             | 5 (100)                  | 6-64                 | 28.8      |
| Hemibarbus longirostris | 4                   | 1 (25.0)                 | -                    | 7.0       |
| Subtotal               | 233                  | 115 (49.4)               | 1-64                 | 5.7       |
| **2017**               |                      |                          |                      |           |
| Pungtungia herzi       | 53                   | 24 (45.3)                | 1-73                 | 12.9      |
| Zacco platypus         | 38                   | 6 (15.8)                 | 1-15                 | 7.7       |
| Acheilognathus majaclusus | 37                | 27 (73.0)                | 1-52                 | 14.9      |
| Acheilognathus yamatsutae | 31                | 23 (74.2)                | 1-28                 | 5.6       |
| Zacca temminckii       | 30                   | 6 (20.3)                 | 1-7                  | 3.0       |
| Carassius auratus      | 28                   | 1 (3.6)                  | -                    | 1.0       |
| Acanthorhodeus gracilis | 21                 | 10 (47.6)                | 16-253               | 57.1      |
| Zacco koreanus         | 20                   | 9 (45.0)                 | 1-14                 | 7.7       |
| Coreperca herzi        | 18                   | 1 (5.6)                  | -                    | 1.0       |
| Squalidus japonicus coreanus | 15             | 15 (100)                 | 1-108                | 31.4      |
| Hemibarbus labeo       | 8                    | 2 (25.0)                 | 2-5                  | 3.5       |
| Hemibarbus longirostris | 7                   | 4 (57.1)                 | 1-2                  | 1.3       |
| Acheilognathus rhombeus | 7                   | 3 (42.9)                 | 4-50                 | 24.0      |
| Acheilognathus koreensis | 5                  | 3 (60.0)                 | 2-33                 | 14.0      |
| Squalidus gracilis majmiae | 3                 | 1 (33.3)                 | -                    | 1.0       |
| Acanthorhodeus macropterus | 2                  | 2 (100)                  | 8-9                  | 8.5       |
| Subtotal               | 322                  | 137 (42.4)               | 1-253                | 15.8      |
| **Total**              | 1,578                | 811 (51.4)               | 1-253                | 9.8       |
Table 4. Infection status of *Clinostomum complanatum* metacercariae by the fish groups from Yangcheon in Sancheong-gun, Gyeongsangnam-do, Korea

| Fish examined                              | No. of fish examined | No. of fish infected (%) | No. of CcMc detected | Range | Average |
|--------------------------------------------|----------------------|--------------------------|----------------------|-------|---------|
| *Squalidus japonicus coreanus*             | 77                   | 76 (98.7)                | 1-108                | 23.6  |
| *Squalidus chinakaisis*                    | 51                   | 51 (100)                 | 1-143                | 32.6  |
| *Squalidus gracilis majimae*               | 3                    | 1 (33.3)                 | -                    | 1.0   |
| Subtotal                                   | 131                  | 128 (97.7)               | 1-143                | 27.0  |
| *Hemibarbus longirostris*                  | 77                   | 20 (26.0)                | 1-7                  | 2.4   |
| *Hemibarbus labo*                          | 8                    | 2 (25.0)                 | 2-5                  | 3.5   |
| Subtotal                                   | 85                   | 22 (25.9)                | 1-7                  | 2.5   |
| *Acheilognathus majusculus*                | 194                  | 132 (68.0)               | 1-52                 | 7.9   |
| *Acheilognathus koreensis*                 | 96                   | 82 (85.4)                | 1-74                 | 7.6   |
| *Acheilognathus rhombeus*                  | 35                   | 23 (65.7)                | 1-50                 | 7.0   |
| *Acheilognathus yamatsutae*                | 58                   | 41 (70.7)                | 1-28                 | 4.8   |
| *Acanthorhodeus gracilis*                  | 41                   | 15 (36.6)                | 1-253                | 39.1  |
| *Acanthorhodeus macropterus*               | 19                   | 3 (15.8)                 | 1-9                  | 6.0   |
| Subtotal                                   | 443                  | 296 (66.8)               | 1-253                | 8.9   |
| *Zacco platypus*                           | 190                  | 80 (42.1)                | 1-22                 | 3.9   |
| *Zacco koreanus*                           | 174                  | 84 (48.3)                | 1-22                 | 3.6   |
| *Zacco temminckii*                         | 91                   | 16 (17.6)                | 1-12                 | 3.4   |
| Subtotal                                   | 455                  | 180 (39.6)               | 1-22                 | 3.7   |
| *Pungtungia herzi*                         | 333                  | 173 (52.0)               | 1-73                 | 6.6   |
| *Carassius auratus*                        | 70                   | 7 (10.0)                 | 1-5                  | 2.0   |
| *Coreoperca herzi*                         | 48                   | 3 (6.3)                  | -                    | 1.0   |
| *Odontobutis platycephala*                 | 13                   | 2 (15.4)                 | 5-10                 | 7.5   |
| Total                                      | 1,578                | 811 (51.4)               | 1-253                | 9.8   |

Table 5. Infection status of *Clinostomum complanatum* metacercariae by the fish species and survey area in the water systems of Nakdong-gang in the east coast of Korea

| Locality and fish sp.                      | No. of fish examined | No. of fish infected (%) | No. of CcMc detected | Range | Average |
|--------------------------------------------|----------------------|--------------------------|----------------------|-------|---------|
| Whangpicheon in Uljin-gun                  |                      |                          |                      |       |         |
| *Pungtungia herzi*                         | 47                   | 3 (6.4)                  | 2-6                  | 3.7   |
| *Squalidus gracilis majimae*               | 6                    | 1 (16.7)                 | -                    | 6.0   |
| Subtotal                                   | 53                   | 4 (7.6)                  | 2-6                  | 4.3   |
| Osipcheon in Yeongdeok-gun                 |                      |                          |                      |       |         |
| *Squalidus gracilis majimae*               | 14                   | 5 (35.7)                 | 1-5                  | 2.2   |
| Checkgwacheon in Ulju-gun                  |                      |                          |                      |       |         |
| *Zacco temminckii*                         | 50                   | 1 (2.0)                  | -                    | 1.0   |
| *Pungtungia herzi*                         | 47                   | 13 (27.7)                | 1-13                 | 3.8   |
| *Rhynchocypris oxycephalus*                | 20                   | 1 (5.0)                  | -                    | 3.0   |
| *Acheilognathus rhombeus*                  | 20                   | 5 (25.0)                 | 1-4                  | 1.8   |
| *Acanthorhodeus gracilis*                  | 17                   | 7 (41.2)                 | 3-9                  | 4.9   |
| *Zacco platypus*                           | 15                   | 2 (13.3)                 | -                    | 1.0   |
| Subtotal                                   | 169                  | 29 (17.2)                | 1-13                 | 3.4   |
| Taehwa-gang in Ulju-gun                    |                      |                          |                      |       |         |
| *Carassius auratus*                        | 14                   | 1 (7.1)                  | -                    | 1.0   |
| Total                                      | 250                  | 39 (15.6)                | 1-13                 | 3.3   |
Cheon in Sancheong-gun, Gyeongsangnam-do, Korea.

Two types of CcMc were reported in India. Non-encysted ones are usually found in the body cavity of Trichogaster fasciatus with high infection rates [22]. They are known to be the pathogenic agents in the viscera and musculature of many fish species [23]. Meanwhile, encysted ones are detected in the body cavity of Channa punctatus and they also act as the etiologic agents in their habitat organs of fish [24]. However, we only found non-encysted CcMc in this study. Generally, we have been thought that the cyst wall of CcMc is so feeble and then it is easily bursted by the process of artificial digestion method. The further studies are needed whether CcMc is to be non-encysted or excysted in the digestion process of fish meat in Korea.

Several species of ardeid birds have been reported as the natural definitive hosts of C. complanatum in the world. Even in Japan, C. complanatum adults were recovered from 4 species of wild birds, i.e., Nycticorax nycticorax, Ardea cinerea, Egretta garzetta, and E. intermedia, in Tottori prefecture [25]. However, only chick was proved as the experimental definitive host in Korea [9]. Accordingly, we should pay attention to the recovery of C. complanatum adults in the survey of wild birds.

More than 50 species have been reported as the members of genus Clinostomum by a variety of authors, even though their taxonomic validity is quite controversial. Among them, C. complanatum is the type-species and the most widely distributed in the world including Korea [3,9,20-24]. It is questionable that every Clinostomum specimens collected in various species of fish from various geographical areas of Korean peninsula are CcMc, although specimens from human cases and experimental chick were already named as C. complanatum. The molecular study on the Clinostomum specimens from various origins should be carried out in the near future in Korea.

Conclusively, it is confirmed that the variety of fish species act as the second intermediate hosts of C. complanatum in the water systems of Nakdong-gang, and the endemicities of CcMc are more or less different by the fish group and species from Yangcheon, most endemic area, in Sancheong-gun, Gyeongsangnam-do, Korea.

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**CONFLICT OF INTEREST**

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

**REFERENCES**

1. Kitagawa N, Oda M, Totoki T, Washizaki S, Oda M, Kifune T. Lidocaine spray used to capture a live Clinostomum parasite causing human laryngitis. Am J Otolaryngol 2003; 24: 341-343.
2. Hara H, Miyauchi Y, Tahara S, Yamashita H. Human laryngitis caused by Clinostomum complanatum. Nagoya J Med Sci 2014; 76: 181-185.
3. Chung DI, Moon CH, Hong HH, Choi DW, Lim DK. The first human case of Clinostomum complanatum (Trematoda: Clinostomidae) infection in Korea. Korean J Parasitol 1995; 33: 219-223.
4. Park CW, Kim JS, Joo HS, Kim J. A human case of Clinostomum complanatum infection in Korea. Korean J Parasitol 2009; 47: 401-404.
5. Jung SC, Oh HJ, Kim DM, Park JH. A case of pharyngitis caused by Clinostomum complanatum. Korean J Otorhinolaryngol-Head Neck Surg 2015; 58: 61-63 (in Korean).
6. Lee GS, Park SW, Kim J, Seo KS, You KW, Chung JH, Moon HC, Hong GY. A case of endoscopically treated laryngopharyngitis resulting from Clinostomum complanatum infection. Korean J Gastroenterol 2017; 69: 177-180.
7. Song HB, Choi MH, Chung EJ. Human laryngeal infection by Clinostomum complanatum. Am J Trop Med Hyg 2018; 98: 7-8.
8. Kim HJ, Cho SW, Oh HR, Byeon HK. A case of unexpected Clinostomum complanatum infection initially presenting as foreign body in pharynx. Korean J Parasitol 2019; 57: 175-177.
9. Chung DI, Kong HH, Moon CH. Demonstration of the second intermediate hosts of Clinostomum complanatum in Korea. Korean J Parasitol 1995; 33: 305-312.
10. Chung DI, Kong HH, Joo CY. Radix auricularia coreana: Natural snail host of Clinostomum complanatum in Korea. Korean J Parasitol 1998; 36: 1-6.
11. Rim HJ, Kim KH, Joo KH, Kim SJ, Eom KS, Chung MS. The infestation status and changing patterns of human infecting metacercariae in freshwater fish in Kyongsang-do and Kyonggi-do, Korea. Korean J Parasitol 1996; 34: 95-105.
12. Sohn WM, Na BK, Cho SH, Lee SW, Choi SB, Seok WS. Trematode metacercariae in freshwater fish from Water Systems of Hantangang and Imjingang in Republic of Korea. Korean J Parasitol 2015; 53: 289-298.
13. Sohn WM, Na BK, Park MY, Kim CH, Hwang MA, No KW, Yoon KB, Lim HC. Prevalence of Clonorchis sinensis metacercariae in fish from water systems of Seomjin-gang (River). Korean J Parasitol 2017; 55: 305-312.
14. Sohn WM, Na BK, Cho SH, Park MY, Kim CH, Hwang MA, No KW, Yoon KB, Lim HC. Prevalence of Clonorchis sinensis metacercariae in freshwater fish from Wicheon Stream in Gunwi-gun, Gyeongsangbuk-do, Korea. Korean J Parasitol 2018; 56: 41-48.
15. Yoon KB, Lim HC, Jeon DY, Park S, Cho SH, Ju JW, Shin SS, Na BK, Sohn WM. Infection status with Clonorchis sinensis metacercariae in fish from Tamjin-gang (River) in Jeollanam-do, Republic of Korea. Korean J Parasitol 2018; 56: 183-188.
16. Sohn WM, Na BK, Cho SH, Ju JW, Kim CH, Yoon KB. Infection status with Metagonimus spp. metacercariae in fishes from Seomjin-gang and Tamjin-gang in Republic of Korea. Korean J Parasitol 2018; 56: 351-358.
17. Sohn WM, Na BK, Cho SH, Ju JW, Lee SW, Seok WS. Infections with zoonotic trematode metacercariae in yellowfin goby, Acanthogobius flavimanus, from coastal areas of Republic of Korea. Korean J Parasitol 2018; 56: 259-265.
18. Sohn WM, Na BK, Cho SH, Lee WJ. Prevalence and density of digenetic trematode metacercariae in clams and oysters from western coastal regions of the Republic of Korea. Korean J Parasitol 2017; 55: 399-408.
19. Sohn WM, Na BK, Cho SH, Ju JW. Infection status of Isthmiophora hortensis metacercariae in dark sleepers, Odontobutis species, from some water systems of the Republic of Korea. Korean J Parasitol 2018; 56: 633-637.
20. Sohn WM. Fish-borne zoonotic trematode metacercariae in the Republic of Korea. Korean J Parasitol 2009; 47 (suppl): 103-113.
21. Aohagi Y, Shibahara T, Machida N, Yamaga Y, Kagota K. Clinostomum complanatum (Trematoda: Clinostomatidae) in five new fish hosts in Japan. J Wildl Dis 1992; 28: 467-469.
22. Siddiqui AA, Nizami WA. Seasonal population dynamics of the metacercariae of Clinostomum complanatum (Trematoda: Digenia) in relation to sex of the host. Riv Parasitol 1982; 43: 275-279.
23. Kalantan AMN, Arfin M, Nizami WA. Seasonal incidence and pathogenicity of the metacercariae of Clinostomum complanatum in Aphanius dispar. Jpn J Parasitol 1988; 36: 17-23.
24. Shareef PA, Abidi S. Incidence and histopathology of encysted progenet metacercaria of Clinostomum complanatum (Digena: Clinostomidae) in Channa punctatus and its development in experimental host. Asian Pac J Trop Biomed 2012; 2: 421-426.
25. Aohagi Y, Shibahara T, Machida N, Yamaga Y, Kagota K, Hayashi T. Natural infections of Clinostomum complanatum (Trematoda: Clinostomatidae) in wild herons and egrets, Tottori Prefecture, Japan. J Wildl Dis 1992; 28: 470-471.
