

Infection Status of Estuarine Fish and Oysters with Intestinal Fluke Metacercariae in Muan-gun, Jeollanam-do, Korea

Shin-Hyeong Cho¹, In-Sang Kim², Eun-Jung Hwang², Tong-Soo Kim³, Byoung-Kuk Na⁴ and Woon-Mok Sohn^{4,*}

¹Division of Malaria and Parasitic Diseases, National Institute of Health, Centers for Disease Control and Prevention, Osong 363-951, Korea; ²Health Center of Muan-gun, Muan 534-804, Korea; ³Department of Parasitology and Inha Research Institute for Medical Sciences, Inha University College of Medicine, Incheon 400-712, Korea; ⁴Department of Parasitology and Institute of Health Sciences, Gyeongsang National University School of Medicine, Jinju 660-751, Korea

Abstract: The source of human infection with intestinal flukes was surveyed in estuarine fishes, including the dotted gizzard shad, common sea bass, common blackish goby, redlip mullet, black sea bream, and oyster collected from Muan-gun, Jeollanam-do, Korea during August and September 2007. Collected fishes and oysters were artificially digested in pepsin-HCl solution and examined under a stereomicroscope. In 36 shads (*Konosirus punctatus*) and 20 basses (*Lateolabrax japonicus*) examined, *Heterophyopsis continua* metacercariae were found in 58.3% and 100%, and their average numbers were 12.0 and 6.3 per infected fish, respectively. In 34 gobies (*Acanthogobius flavimanus*) examined, metacercariae of *H. continua* were detected in 79.4%, *Stictodora lari* in 97.1%, and *Acanthotrema felis* in 92.1%, and their average numbers were 45.8, 189.3, and 235.3 per infected fish, respectively. In 37 redlip mullets (*Chelon haematocheilus*), *Heterophyes nocens* metacercariae were found in 56.8%, *Pygidiopsis summa* in 94.6%, and *Stictodora fuscata* in 45.9%, and the average metacercarial densities were 17.4, 31.3, and 35.1 per infected fish, respectively. In 30 black sea breams (*Acanthopagrus schlegelii*) and 45 oysters (*Crassostrea gigas*) examined, no metacercariae were detected. From the above results, it has been confirmed that the dotted gizzard shad, common sea bass, common blackish goby, and redlip mullet from Muan-gun, Jeollanam-do, Korea are infected with the metacercariae of heterophyid flukes.

Key words: *Heterophyopsis continua*, *Stictodora lari*, *Acanthotrema felis*, *Heterophyes nocens*, *Pygidiopsis summa*, *Stictodora fuscata*, metacercaria, fish, oyster, Muan-gun

INTRODUCTION

Foodborne intestinal trematodes affect the health of more than 40 million people around the world. About 70 species belonging to 14 families are known to participate in human infections. Among them, heterophyid trematodes (members of the family Heterophyidae) are minute parasites that are clinically important in both intestinal and extraintestinal infections. More than 30 species in 13 genera have been known to infect human beings all over the world [1-4]. In the Republic of Korea (= Korea), 11 species in 8 genera have been reported as human-infecting species of heterophyid flukes. Among

them, 7 species (*Heterophyes nocens*, *Heterophyopsis continua*, *Pygidiopsis summa*, *Stellantchasmus falcatus*, *Stictodora fuscata*, *Stictodora lari*, and *Acanthotrema felis*) are infected by consumption of the raw flesh of estuarine fish [2,5-11].

It has been shown that a lot of residents in south and west coastal areas of Korea are infected with several species of intestinal flukes [12-17]. Estuarine fish, such as *Lateolabrax japonicus*, *Konosirus punctatus*, *Mugil cephalus*, *Chelon haematocheilus*, *Acanthogobius flavimanus*, *Boleophthalmus pectinirostris*, and *Scartelaos* sp., have been reported as the second intermediate host and/or the source of human infection of heterophyid flukes in endemic areas [5-11,18-24]. These fish hosts are sometimes heavily infected with metacercariae of heterophyid flukes, and are popularly eaten raw by many residents in coastal areas. On the other hand, Muan-gun, Jeollanam-do was recently known as a high endemic area of intestinal flukes [25]. However, the infection source of these flukes were partly reported in the surveyed area. Therefore, we performed an epidemiological survey

•Received 7 May 2012, revised 7 June 2012, accepted 7 June 2012.

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

© 2012, Korean Society for Parasitology

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

to know the infection status of metacercariae in estuarine fish and oysters collected from 3 coastal areas in Muan-gun, Jeollanam-do, Korea.

MATERIALS AND METHODS

During August and September 2007, the dotted gizzard shad (*K. punctatus*), common sea bass, (*L. japonicus*), common blackish goby (*A. flavimanus*), redlip mullet (*C. haematocheilus*), black sea bream (*Acanthopagrus schlegeli*), and oyster (*Crassostrea gigas*) were collected in 3 administrative regions of Muan-gun, Jeollanam-do, Korea (Fig. 1). All collected fishes and oysters were transferred to our laboratory (Department of Parasitology, Gyeongsang National University School of Medicine, Jinju, Korea) with ice, measured for length and weight, and examined by artificial digestion method (Table 1). Each fish was finely ground with a mortar with a pestle, or grinder, the ground fish meat was mixed with artificial gastric juice, and the mixture was incubated at 36°C for 2-3 hr. The digested material was filtered with 1×1 mm of mesh, and washed with 0.85% saline until the supernatant became clear. Metacercariae were collected from the sediment under a stereomicroscope, and

categorized according to the measurements and morphological characters. Infection rates and intensities were then calculated.

RESULTS

Heterophyopsis continua metacercariae

Metacercariae of *H. continua* were detected in 21 (58.3%) dotted gizzard shads, *K. punctatus*, 20 (100%) common sea basses, *L. japonicus*, and 27 (79.4%) common blackish gobies, *A. flavimanus*. The average metacercarial density per infected fish was 11.9 in *K. punctatus*, 6.3 in *L. japonicus*, and 45.8 in *A. flavimanus*, respectively. The infection status of 3 fish species by their collection site is depicted in Table 2.

Stictodora lari and *Acanthotrema felis* metacercariae

Besides the metacercariae of *H. continua*, those of *S. lari* and *A. felis* were also detected in *A. flavimanus*. Metacercariae of *S. lari* were detected in 33 (97.1%) gobies, and *A. felis* metacercariae were found in 31 (91.2%) of 34 gobies examined. The average number of metacercariae per infected fish was 189.3 (*S. lari*) and 235.3 (*A. felis*), respectively. The infection status

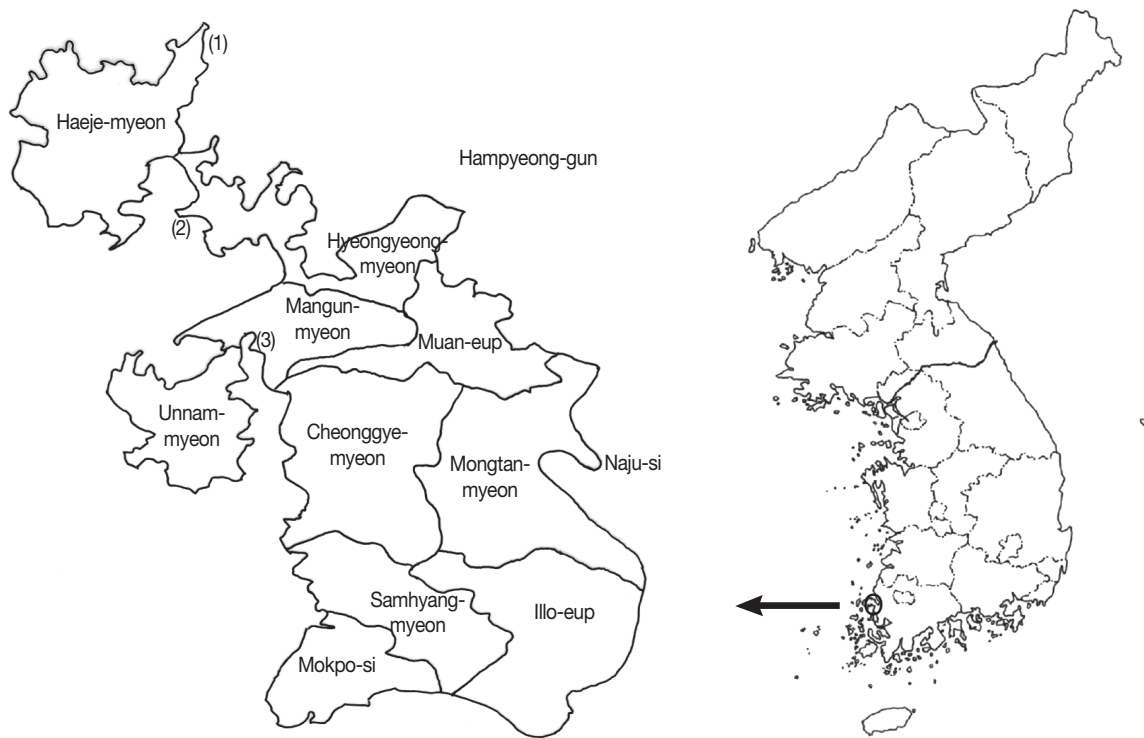


Fig. 1. Surveyed areas (O). 1, Songseok-ri in Haeje-myeon; 2, Oryu-ri in Hyeongyeong-myeon; 3, Piseo-ri in Mangun-myeon, Muan-gun, Jeollanam-do, Korea.

Table 1. Estuarine fish and oysters collected from Muan-gun, Jeollanam-do, Korea

Fish and locality ^a	No. of fish examined	Length (cm)		Weight (g)	
		Range	Average	Range	Average
<i>Konosirus punctatus</i>					
Haeje-myeon	14	17.6-19.9	18.5	41.2-67.4	52.7
Hyeongyeong-myeon	12	17.5-19.0	18.6	42.7-55.7	49.6
Mangun-myeon	10	10.7-18.0	14.3	8.6-52.0	28.8
<i>Lateolabrax japonicus</i>					
Hyeongyeong-myeon	10	18.0-21.0	19.5	61.4-91.7	79.6
Mangun-myeon	10	18.3-21.0	19.4	58.6-100.1	74.0
<i>Acanthogobius flavimanus</i>					
Haeje-myeon	13	11.5-15.3	13.8	10.6-27.0	19.6
Hyeongyeong-myeon	10	13.0-20.7	17.2	13.9-63.8	35.4
Mangun-myeon	11	12.5-18.5	14.7	14.4-43.5	22.4
<i>Chelon haematocheilus</i>					
Haeje-myeon	12	24.5-30.0	27.1	103.3-231.4	157.0
Hyeongyeong-myeon	15	15.0-23.2	18.3	27.9-97.7	50.8
Mangun-myeon	10	14.3-27.5	18.0	20.2-156.9	49.9
<i>Acanthopagrus schlegelii</i>					
Haeje-myeon	10	9.7-14.0	12.2	17.6-48.0	31.8
Hyeongyeong-myeon	10	11.7-20.2	16.5	23.2-68.7	47.8
Mangun-myeon	10	10.2-17.5	14.6	18.8-54.7	40.3
<i>Crassostrea gigas</i>					
Haeje-myeon	15	-	-	17.6-48.0	31.8
Hyeongyeong-myeon	15	-	-	23.2-68.7	47.8
Mangun-myeon	15	-	-	18.8-54.7	40.3

^aSurveyed areas: Songseok-ri in Haeje-myeon; Oryu-ri in Hyeongyeong-myeon; Piseo-ri in Mangun-myeon, Muan-gun, Jeollanam-do, Korea.

Table 2. Infection status of *Heterophyopsis continua* metacercariae in fish collected from Muan-gun, Jeollanam-do, Korea

Fish species and locality	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Konosirus punctatus</i>					
Haeje-myeon	14	8 (57.1)	70	1-56	8.8
Hyeongyeong-myeon	12	9 (75.0)	161	1-88	17.9
Mangun-myeon	10	4 (40.0)	19	2-8	4.8
Total	36	21 (58.3)	250	1-88	11.9
<i>Lateolabrax japonicus</i>					
Hyeongyeong-myeon	10	10 (100)	98	3-35	9.8
Mangun-myeon	10	10 (100)	27	1-8	2.7
Total	20	20 (100)	125	1-35	6.3
<i>Acanthogobius flavimanus</i>					
Haeje-myeon	13	13 (100)	501	1-135	38.5
Hyeongyeong-myeon	10	10 (100)	449	4-165	44.9
Mangun-myeon	11	4 (36.4)	11	1-6	2.8
Total	34	27 (79.4)	961	1-165	45.8

by the fish collection site is shown in Table 3.

***Heterophyes nocens*, *Pygidiopsis summa*, and *Stictodora fuscata* metacercariae**

In the redlip mullet, *C. haematocheilus*, metacercariae of *H.*

nocens, *P. summa*, and *S. fuscata* were detected. *H. nocens* metacercariae were found in 21 (56.8%) mullets, and their average number per infected fish was 17.4. A total of 1,097 *P. summa* metacercariae were collected from 35 (94.6%) mullets. *S. fuscata* metacercariae were detected in 17 (45.9%) of 37 mullets

Table 3. Infection status of *Stictodora lari* and *Acanthotrema felis* metacercariae in *Acanthogobius flavimanus* fish from Muan-gun, Jeollanam-do, Korea

Trematode species and locality	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Stictodora lari</i>					
Haeje-myeon	13	12 (92.3)	1,368	17-377	114.0
Hyeongyeong-myeon	10	10 (100)	2,475	22-572	247.5
Mangun-myeon	11	11 (100)	2,403	5-830	218.5
Total	34	33 (97.1)	6,246	5-830	189.3
<i>Acanthotrema felis</i>					
Haeje-myeon	13	10 (76.9)	509	1-187	50.9
Hyeongyeong-myeon	10	10 (100)	4,322	55-1,156	432.2
Mangun-myeon	11	11 (100)	2,462	8-789	223.8
Total	34	31 (91.2)	7,293	1-1,156	235.3

examined, and their average number per infected fish was 35.1. The infection status by the mullet collection site is shown in Table 4.

Other metacercariae

No metacercariae were detected in 30 black sea breams, *A. schlegeli*, and 45 oysters, *C. gigas*, examined. However, metacercariae of *Gymnophalloides seoi* were found in group examinations of oysters, about 100 oysters each from Haeje-myeon (40 metacercariae of *G. seoi*) and Hyeongyeong-myeon (8 metacercariae of *G. seoi*).

DISCUSSION

By the present study, it has been confirmed that intestinal flukes are prevalent not only in the human definitive host [25] but also in fish intermediate host in Muan-gun, Jeollanam-do, Korea. Chai et al. [12] reported a high egg positive rate (75.0%) of heterophyid flukes among 108 residents in a small coastal village of Muan-gun. Cho et al. [25] detected eggs of heterophyids from 62 (4.9%) of 1,257 residents in Muan-gun, and they recovered 6 species of heterophyid flukes (*H. nocens*, *P. summa*, *S. falcatus*, *S. fuscata*, *S. lari*, and *A. felis*), and *G. seoi* from 9 residents after praziquantel treatment and purgation. In the present study, 6 heterophyid species metacercariae (*H. nocens*, *H. continua*, *P. summa*, *S. fuscata*, *S. lari*, and *A. felis*) were detected from the redlip mullet, common blackish goby, dotted gizzard shad, and common sea bass collected in Muan-gun.

H. nocens is known to be the dominant species of heterophyid among the residents of western and southern coastal ar-

Table 4. Infection status of *Heterophyes nocens*, *Pygidiopsis summa*, and *Stictodora fuscata* metacercariae in *Chelon haematocheilus* fish from Muan-gun, Jeollanam-do, Korea

Trematode species and locality	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Heterophyes nocens</i>					
Haeje-myeon	12	11 (91.7)	196	1-71	17.8
Hyeongyeong-myeon	15	2 (13.3)	67	2-65	33.5
Mangun-myeon	10	8 (80.0)	103	1-62	12.9
Total	37	21 (56.8)	366	1-71	17.4
<i>Pygidiopsis summa</i>					
Haeje-myeon	12	11 (91.7)	356	1-126	32.4
Hyeongyeong-myeon	15	14 (93.3)	318	1-257	22.7
Mangun-myeon	10	10 (100)	423	2-240	42.3
Total	37	35 (94.6)	1,097	1-257	31.3
<i>Stictodora fuscata</i>					
Haeje-myeon	12	10 (83.3)	378	9-113	37.8
Hyeongyeong-myeon	15	7 (46.7)	219	1-57	31.3
Mangun-myeon	10	0	-	-	-
Total	37	17 (45.9)	597	1-113	35.1

eas, including Shinan-gun, Gangjin-gun and Muan-gun (Jeollanam-do), Buan-gun (Jeollabuk-do), and Sacheon-si (Gyeong-sangnam-do), Korea [12-16,25]. For the second intermediate host of this fluke, several species of estuarine fish (*M. cephalus*, *C. haematocheilus*, *A. flavimanus*, *B. pectinirostris*, and *Scartelaos* sp.) have been reported [7,18,22]. In the present study, *H. nocens* metacercariae were detected only in the redlip mullet, *C. haematocheilus*. Their infection rate (56.8%) and intensity (17.4 metacercariae per infected fish) were not so high, compared to high worm burdens in human infection cases [12,25]. This finding suggests that residents in endemic areas habitually eat raw flesh of mullets, and worms may be accumulated by repeated infections.

The common blackish goby, *A. flavimanus*, is a suitable fish host for 6 species of heterophyid flukes (*H. nocens*, *H. continua*, *P. summa*, *S. fuscata*, *S. lari*, and *A. felis*) in Korea [10,11,18,19]. In the present study, 3 species of metacercariae (*H. continua*, *S. lari*, and *A. felis*) were detected; their infection rates (79.4, 97.1, and 91.2%) were relatively high and the intensity was about 46, 189, and 235 metacercariae per infected fish, respectively. Sohn et al. [24] also detected 3 species of metacercariae (*H. continua*, *Stictodora* spp., and *H. nocens*) in 15 gobies from Haeje-myeon in Muan-gun. In the case of *H. continua* metacercariae in the goby from Muan-gun, the infection rate was all 100%, and the intensity was slightly higher in the present study (38.5 metacercariae) than in Sohn et al. [24] (23.3 metacercariae).

In addition, by the present study, it is confirmed that *A. flavimanus* briskly act as a second intermediate host for *A. felis* in Muan-gun, Jeollanam-do, Korea [11].

No metacercariae were detected in 30 black sea breams and 45 oysters examined individually. However, *G. seoi* metacercariae were found in group examination of about 100 oysters from Haeje-myeon and Hyeongyeong-myeon. These findings provide a background for positive adult worm recovery in residents [12,25], and suggest that oysters from Muan-gun, Jeollanam-do act as the source of *G. seoi* infection, although their metacercarial density is very low.

Metacercarial examination in the second intermediate host, in combination with a survey on adult worms in humans and also on larvae in the snail intermediate host, can be a useful index in the epidemiology of trematodes in a particular area. However, in the case of heterophyid trematodes, fecal examinations are not successful to determine the infection status in humans and reservoir hosts, since the egg production amount is too small, and the first intermediate host is not well known. Therefore, we investigated the infection status of estuarine fish with metacercariae in order to provide epidemiological information for heterophyid flukes in Muan-gun, Jeollanam-do. Conclusively, the present study suggested that those who consume raw estuarine fish from Muan-gun are at a high risk of infection with heterophyid flukes.

ACKNOWLEDGMENTS

This study was supported by an Anti-Communicable Diseases Control Program of the National Institute of Health (NIH 348-6111-215), Ministry of Health and Welfare, the Republic of Korea. We thank Miss A-Ra Cho and Hae-In Ryu, Department of Parasitology, Gyeongsang National University School of Medicine, Jinju, Korea, for their help in fish examinations.

REFERENCES

- Chai JY. Intestinal flukes. In food-borne parasitic zoonoses: Fish and plant-borne parasites. World Class Parasites, Vol 11. New York, USA. Springer. 2007. p 53-115.
- Chai JY, Lee SH. Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int 2002; 51: 129-154.
- Africa CM, de Leon W, Garcia EY. Visceral complications in intestinal heterophyidiasis of man. Acta Medica Philippina Monogr Ser 1940; No. 1: 1-325.
- Cho SY, Kim SI, Earm YE, Ho WK. A preliminary observation on watery content of small intestine in *Metagonimus yokogawai* infected dogs. Korean J Parasitol 1985; 23: 175-179.
- Chun SK. A study on some trematodes whose intermediate host are brackish water fish. (1) The life history of *Heterophyes continuous*, the intermediate host of which is *Laterolabrax japonicus*. Bull Pusan Fish Coll 1960; 3: 40-44 (in Korean).
- Chun SK. A study on some trematodes whose intermediate host are brackish water fish. (II) The life history of *Pygidiopsis summa*, the intermediate host of which is *Mugil cephalus*. Bull Pusan Fish Coll 1963; 5: 1-5 (in Korean).
- Seo BS, Cho SY, Chai JY, Hong ST. Studies on intestinal trematodes in Korea II. Identification of the metacercariae of *Heterophyes heterophyes nocens* in mullets of three southern coastal areas. Seoul J Med 1980; 21: 30-38.
- Chai JY, Sohn WM. Identification of *Stellantchasmus falcatus* metacercariae encysted in mullets in Korea. Korean J Parasitol 1988; 26: 65-68.
- Chai JY, Park SK, Hong SJ, Choi MH, Lee SH. Identification of *Stictodora lari* (Heterophyidae) metacercariae encysted in the brackish water fish, *Acanthogobius flavimanus*. Korean J Parasitol 1989; 27: 253-259.
- Sohn WM, Chai JY, Lee SH. *Stictodora fuscatum* (Heterophyidae) metacercariae encysted in gobies, *Acanthogobius flavimanus*. Korean J Parasitol 1994; 32: 143-148.
- Sohn WM, Han ET, Seo M, Chai JY (2003) Identification of *Acanthotrema felis* (Digenea: Heterophyidae) metacercariae encysted in the brackish water fish *Acanthogobius flavimanus*. Korean J Parasitol 2003; 41: 101-105.
- Chai JY, Kim IM, Seo M, Guk SM, Kim JL, Sohn WM, Lee SH. A new endemic focus of *Heterophyes nocens*, *Pygidiopsis summa*, and other intestinal flukes in a coastal area of Muangun, Chollanam-do. Korean J Parasitol 1997; 35: 233-238.
- Chai JY, Nam HK, Kook J, Lee SH. The first discovery of an endemic focus of *Heterophyes nocens* (Heterophyidae) infection in Korea. Korean J Parasitol 1994; 32: 157-161.
- Chai JY, Song TE, Han ET, Guk SM, Park YK, Choi MH, Lee SH. Two endemic foci of heterophyids and other intestinal fluke infections in southern and western coastal areas in Korea. Korean J Parasitol 1998; 36: 155-161.
- Chai JY, Park JH, Han ET, Shin EH, Kim JL, Guk SM, Hong KS, Lee SH, Rim HJ. Prevalence of *Heterophyes nocens* and *Pygidiopsis summa* infections among residents of the western and southern coastal islands of the Republic of Korea. Am J Trop Med Hyg 2004; 71: 617-622.
- Park JH, Kim JL, Shin EH, Guk SM, Park YK, Chai JY. A new endemic focus of *Heterophyes nocens* and other heterophyid infections in a coastal area of Gangjin-gun, Jeollanam-do. Korean J Parasitol 2007; 45: 33-38.
- Chai JY, Hong SJ, Lee SH, Seo BS. *Stictodora* sp. (Trematoda: Heterophyidae) recovered from a man in Korea. Korean J Parasitol 1988; 26: 127-132.
- Seo BS, Hong ST, Chai JY, Cho SY. Studies on intestinal trematodes in Korea IV. Geographical distribution of *Pygidiopsis* and

- Heterophyes* metacercariae. Seoul J Med 1981; 22: 236-242.
19. Seo BS, Lee SH, Chai JY, Hong SJ. Studies on intestinal trematodes in Korea XIII. Two cases of natural human infection by *Heterophysis continua* and the status of metacercarial infection in brackish water fishes. Korean J Parasitol 1984; 22: 51-60.
 20. Sohn WM, Han GG, Kho WG, Chai JY, Lee SH. Infection status with the metacercariae of heterophyid flukes in the brackish water fish from Haenam-gun, Chollanam-do, Korea. Korean J Parasitol 1994; 32: 163-169 (in Korean).
 21. Sohn WM, Moon BC. Infection status with the metacercariae of heterophyid trematode in mullet and goby collected from western coastal areas of Cholla-do, Korea. J Biomed Lab Sci 2001; 7: 31-37.
 22. Sohn WM, Kim JA, Cho HJ. Two species of goby, *Boleophthalmus pectinirostris* and *Scartelaos* sp., as the new second intermediate host of heterophyid flukes in Korea. Korean J Parasitol 2005; 43: 161-164.
 23. Kim DG, Kim TS, Cho SH, Song HJ, Sohn WM. Heterophyid metacercarial infections in brackish water fishes from Jinju-man (Bay), Kyongsangnam-do, Korea. Korean J Parasitol 2006; 44: 7-13.
 24. Sohn WM, Na BK, Cho SH. *Echinostoma hortense* and heterophyid metacercariae encysted in yellowfin goby, *Acanthogobius flavimanus*, from Shinan-gun and Muan-gun (Jeollanam-do), Korea. Korean J Parasitol 2009; 47: 307-310.
 25. Cho SH, Cho PY, Lee DM, Kim TS, Kim IS, Hwang EJ, Na BK, Sohn WM. Epidemiological survey on the infection of intestinal flukes in residents of Muan-gun, Jeollanam-do, the Republic of Korea. Korean J Parasitol 2010; 48: 133-138.