



Prevalence and Density of Digenetic Trematode Metacercariae in Clams and Oysters from Western Coastal Regions of the Republic of Korea

Woon-Mok Sohn^{1,*}, Byoung-Kuk Na¹, Shin-Hyeong Cho², Won-Ja Lee²

¹Department of Parasitology and Tropical Medicine, and Institute of Health Sciences, Gyeongsang National University School of Medicine, Jinju 52727, Korea; ²Division of Malaria and Parasitic Diseases, National Research Institute of Health, Centers for Disease Control and Prevention, Osong 28159, Korea

Abstract: A survey was performed to know the recent infection status of digenetic trematode metacercariae in clams and oysters from 4 sites in western coastal regions of the Republic of Korea (= Korea). Four species of clams (*Mactra veneriformis*, *Ruditapes philippinarum*, *Cyclina sinensis*, and *Saxidomus purpuratus*) were collected from Taeon-gun, Chungcheongnam-do (Province), Buan-gun (County) and Gochang-gun, Jeollabuk-do, and oysters, *Crassostrea gigas*, from Shinan-gun, Jeollanam-do were transferred to our laboratory on ice and examined by the artificial digestion method. The metacercariae of *Himasthla alincia* were detected in 3 species of clams, *M. veneriformis*, *R. philippinarum*, and *C. sinensis* from the 3 surveyed areas. The positive rate and the mean density per clam infected were 98.9% (30.8 metacercariae) in *M. veneriformis*, 60.0% (5.0) in *R. philippinarum*, and 96.0% (28.4) in *C. sinensis*. The positive rate (mean density) of *Acanthoparyphium tyosenense* metacercariae in *M. veneriformis* was 50.0% (2.1) from Taeon-gun and 70.0% (2.8) from Gochang-gun. The metacercariae of *Parvatrema* spp. were detected in *M. veneriformis* and *R. philippinarum* from Taeon-gun and Gochang-gun; the positive rate (mean density) was 63.3% (4,123) and 50.0% (19) in *M. veneriformis*, and 6.7% (126) and 100% (238) in *R. philippinarum* from the 2 regions, respectively. The metacercariae of *Gymnophalloides seoi* were detected in all 30 oysters from Shinan-gun, and their average density per oyster was 646. From the above results, it has been confirmed that more than 3 species of metacercariae are prevalent in clams from the western coastal regions, and *G. seoi* metacercariae are still prevalent in oysters from Shinan-gun, Jeollanam-do, Korea

Key words: *Himasthla alincia*, *Acanthoparyphium tyosenense*, *Parvatrema* sp., clam, oyster

INTRODUCTION

Digenetic trematodes are a relatively large trematode group comprising of more than 2,500 nominal genera. It commonly has 3 hosts (2 intermediate and 1 definitive) in the life cycle. Various kind of animals, i.e., mollusc, fish, amphibian, reptilian, and arthropod, act as the second intermediate hosts, which retained the infective larvae, i.e., metacercariae. Especially, it has been known that some species of digenetic trematode metacercariae are detected in molluscan hosts, the clam and oyster [1-3]. In the Republic of Korea (= Korea), some species of gymnophallid metacercariae were detected from the inter-

tidal clams and oysters [4-11]. Some species of echinostomatid metacercariae were also found in the brackish water gastropods [12-16].

Yu et al. [4] first detected *Parvatrema duboisi* (syn. *P. timondavidi*) metacercariae from Manila clams, *Ruditapes philippinarum*, and Sohn et al. [10] recorded *Parvatrema chaili* n. sp. (Digenea: Gymnophallidae), which were recovered from mice experimentally infected with the metacercariae collected from the surf-clam *Mactra veneriformis*. Lee et al. [5] first found that the oyster, *Crassostrea gigas*, is the second intermediate host (the source of human infection) of *Gymnophalloides seoi*. Kim and Yun [9] surveyed on larval trematodes in 3 species of bivalves, *Corbicula japonica*, *Simonovacula constricta*, and *R. philippinarum*, from some sites in the Yellow Sea. Chai et al. [11] reported a new gymnophallid trematode, *Meiogymnophallus simonovaculae* n. sp., recovered from the small intestines of mice infected with metacercariae from the razor clam, *S. constricta*. On the other hand, Kim and Chun [12] detected *Himasthla kususigi*

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

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metacercariae in the clam, *Meretrix lusori*. Kim [13] also found 2 species of echinostomatid metacercariae, *H. kusiasigi* and *Acanthoparyphium tyosenense*, from 3 species of intertidal clams, i.e., *M. veneriformis*, *Cyclina sinensis*, and *Solen strictus*. After then, Chai et al. [14] discovered human infections with *A. tyosenense*, and they also investigated the metacercarial infections of this fluke in 5 species of bivalves, i.e., *M. veneriformis*, *Solen grandis*, *Meretrix petechialis*, *C. sinensis*, and *Scapharca broughtonii*, and a marine snail, *Neverita bicolor*, to survey on the sources of human infections. Han and Chai [15] reported for the first time that *M. veneriformis* act as the second intermediate host of *Acanthoparyphium marilae*.

The infection status of *Parvatremas* sp. metacercariae was investigated in *R. philippinarum* collected from southern coastal areas of Korea [6]. Lee et al. [7] performed a nationwide survey of naturally produced oysters to know the infection status with *G. seoi* metacercariae. Most of the previous studies were performed to identify the specific trematode species from the limited host animals and areas [4-16]. However, the metacercarial infection status of intertidal bivalves collected from the western coastal regions has not been widely and systematically examined. Therefore, we investigated the infection status of 5 species of bivalves from 4 local sites in the western coast of Korea with the trematode metacercariae.

MATERIALS AND METHODS

Subjected samples by surveyed areas

In July 2013, total 4 species of clams (*Mactra veneriformis*, *Ruditapes philippinarum*, *Cyclina sinensis* and *Saxidomus purpuratus*) were collected from 3 western coastal regions (Fig. 1), i.e., Taean-gun (latitude: 36.4710; longitude: 126.3408) in Chungcheongnam-do, Buan-gun (35.0727; 126.0734) and Gochang-gun (35.5009; 126.4907) in Jeollabuk-do, Korea. A total of 90 *M. veneriformis* were collected in all 3 regions, Taean-gun (n=30), Buan-gun (n=20), and Gochang-gun (n=40). Two species of clams, *R. philippinarum* (n=25) and *C. sinensis* (n=25), were also collected with the same number in 2 regions, Taean-gun (n=15) and Gochang-gun (n=10), respectively. In Buan-gun, 10 *S. purpuratus* were collected. To investigate the recent infection status of *G. seoi* metacercariae, oysters, *Crassostrea gigas*, were collected in July 2013 from a tidal flat of Aphae-myeon (Township) in Shinan-gun (34.0839; 126.3508), Jeollanam-do, a well-known endemic area of this fluke [16].



Fig. 1. Surveyed areas in ① Taean-gun (latitude: 36.4710; longitude: 126.3408), Chungcheongnam-do (CN); ② Buan-gun (35.0727; 126.0734) and ③ Gochang-gun (35.5009; 126.4907), Jeollabuk-do (JB); ④ Shinan-gun (34.0839; 126.3508), Jeollanam-do (JN), Korea.

Examination of the metacercarial infection status in clams and oysters

All collected clams and oysters were transferred on ice to the laboratory of the Department of Parasitology and Tropical Medicine, Gyeongsang National University School of Medicine, Jinju, Korea. After species identification, the bivalves were individually opened with a knife. Each opened sample (the animal part and 2 shells) was mixed with artificial gastric juice, and the mixture was incubated at 36°C for 1-2 hr with occasional stirring (at 15-20 min interval). The digested material was filtered through a 1 × 1 mm mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined under a stereomicroscope. Each species of metacercariae were separately collected by the general feature, and they were counted to get hold of the infection rate (%) and density (no. of metacercariae per clam infected) by clam species.

Observation of metacercariae and experimental infection to animals

Some representative metacercariae collected with a stereo-

microscope were morphologically observed under a light microscope equipped with a micrometer (OSM-4, Olympus, Tokyo, Japan) to identify their exact species. Especially, the metacercariae of *Parvatrema* spp. detected in *M. veneriformis* from Taean-gun were fixed with 10% formalin under a cover slip, and then stained with Semichon's acetocarmine to observe their morphological characteristics. The collected metacercariae were orally infected with a gavage needle to chicks and hamster to obtain adult worms. Two species of echinostomes, *Himasthla alincia* and *Acanthoparyphium tyosenense*, were recovered in the small intestines of chicks at 2 weeks after infection. Many specimens of *Parvatrema* spp. were also recovered in the small intestines of hamsters at day 5 after the infection. The experimental animals, hamsters and chicks, were treated according to the guidelines of Institutional Animal Care and Use Committee (IACUC) in Gyeongsang National University. The recovered worms were fixed with 10% formalin under a cover glass pressure, and then stained with Semichon's acetocarmine to observe their morphological characteristics.

RESULTS

Infection status of *M. veneriformis* with trematode metacercariae

More than 3 species of metacercariae, i.e., *Himasthla alincia*, *A. tyosenense*, and *Parvatrema* spp., were detected in *M. veneriformis*. The metacercariae of *H. alincia* were found in 98.9% clams, and their mean density was 30.8 per clam infected. The metacercariae of *A. tyosenense* were detected in 47.8% clams, and their mean density was 2.5 per clam infected. *Parvatrema* spp. metacercariae were found in 43.3% clams, and their mean density was 2,018 per clam infected. The metacercarial infection status by the surveyed area is designated in detail in Table 1.

Table 1. Infection status of clams, *Macraa veneriformis*, from 3 western coastal regions of Korea with trematode metacercariae

Locality and trematode species	No. (%) of clams infected	No. of metacercariae detected		
		Total	Range	Average
Taeon-gun (n=30)				
<i>Himasthla alincia</i>	30 (100)	1,352	4-120	45.1
<i>Acanthoparyphium tyosenense</i>	15 (50.0)	31	1-7	2.1
<i>Parvatrema</i> sp.	19 (63.3)	78,331	7-18,900	4,123
Buan-gun (n=20)				
<i>Himasthla alincia</i>	20 (100)	480	2-70	24.0
Gochang-gun (n=40)				
<i>Himasthla alincia</i>	39 (97.5)	912	6-174	23.4
<i>Acanthoparyphium tyosenense</i>	28 (70.0)	77	1-8	2.8
<i>Parvatrema</i> sp.	20 (50.0)	382	4-43	19.1

Infection status of *R. philippinarum* with trematode metacercariae

More than 2 species of metacercariae, i.e., *H. alincia* and *Parvatrema* spp., were detected from *R. philippinarum*. The metacercariae of *H. alincia* were found in 80.0% and 30.0% clams from Taean-gun and Gochang-gun, and their mean densities were 5.8 and 1.7 per clam infected, respectively. *Parvatrema* spp. metacercariae were detected in 6.7% and 100% clams from Taean-gun and Gochang-gun, and their mean densities were 126 and 238 per clam infected, respectively (Table 2).

Infection status of *C. sinensis* and *S. purpuratus* with trematode metacercariae

Only 1 species, *H. alincia*, was detected from *C. sinensis* clams. The metacercariae were found in 93.3% and 100% clams from Taean-gun and Gochang-gun, and their mean densities were 5.5 and 60.5 per clam infected, respectively (Table 3). No metacercariae were found in 10 *S. purpuratus* collected in Buan-gun, Jeollabuk-do.

Infection status of oysters with *G. seoi* metacercariae

The metacercariae of *G. seoi* were detected in all 30 oysters

Table 2. Infection status in estuarine clams, *Ruditapes philippinarum*, from 2 western coastal regions of Korea with trematode metacercariae

Locality and trematode species	No. (%) of clams infected	No. of metacercariae detected		
		Total	Range	Average
Taeon-gun (n=15)				
<i>Himasthla alincia</i>	12 (80.0)	70	1-11	5.8
<i>Parvatrema</i> sp.	1 (6.7)	126	-	126
Gochang-gun (n=10)				
<i>Himasthla alincia</i>	3 (30.0)	5	1-2	1.7
<i>Parvatrema</i> sp.	10 (100)	2,377	80-615	238

(100%) collected from Aphae-myeon in Shinan-gun, Jeollanam-do, and their density was 646 per oyster in average. The metacercarial density by the weight group of oysters is shown in detail in Table 4.

Morphology of *H. alincia* metacercariae and adults

Metacercariae (Fig. 2A): Cyst oval or round, 240-257 (249) × 232-250 (242) in size with 5-9 (7) thick cyst wall, had a head collar with 31 collar spines that included 4 lateral end-group spines on each side, and 2 excretory ducts filled with numerous fine granules.

Adults (Fig. 2B): Body markedly elongate, maximum width at the level of the anterior testis. Head crown distinct, bearing 31 collar spines arrange in a single uninterrupted row, with 4 end group spines on each side of ventral corners (Fig. 2C). Oral

sucker small, subterminal. Prepharynx very short. Pharynx subglobular. Esophagus short. Ceca bifurcating near the anterior margin of the ventral sucker, terminating blindly near the posterior extremity. Ventral sucker slightly elliptical. Sucker ratio (oral sucker:ventral sucker) approximately 1:2.93. Cirrus sac elongate, dorsal to ventral sucker, containing seminal vesicle, prostate gland cells, and a cirrus. Ovary spherical, on the median or slightly dextral. Mehlis' gland located between the ovary and the anterior margin of the anterior testis. Two testes oval to elliptical, tandem, intercecal, near the posterior end of the body. Vitellaria follicular, distributing laterally from anterior 1/3 of the body to near the posterior end. Uterus with eggs intercecal, from the Mehlis' gland to the anterior 2/3 of the body. Eggs operculate, golden yellow, 93-108 (100) by 55-63 (60). Dimensions of each organ are revealed in detail in Table 5.

Table 3. Infection status of estuarine clams, *Cyclina sinensis*, from 2 western coastal regions of Korea of trematode metacercariae

Locality and trematode species	No. (%) of clams infected	No. of metacercariae detected		
		Total	Range	Average
Taeon-gun (n=15)				
<i>Himasthla alincia</i>	14 (93.3)	77	1-17	5.5
Gochang-gun (n=10)				
<i>Himasthla alincia</i>	10 (100)	605	10-21	360.5

Table 4. Infection status of *Gymnophalloides seoi* metacercariae in oysters from Shinan-gun, Jeollanam-do

Weight of oysters examined	No. of metacercariae detected		
	Total	Range	Average
Below 30 g (n=12)	4,347	70-1,083	362
31-40 g (n=9)	4,394	126-2,001	488
Over 41 g (n=9)	10,636	27-4,704	1,181
Total (n=30)	19,377	27-4,704	646

Table 5. Comparison of the dimensions of *Himasthla alincia* adults with those of previous studies

Organs	Present study (2017) ^a Range (average)	Han et al. [15] ^b Range (average)	Kim and Chun [11] ^c Range
Body length (BL)	5,625-8,125 (6,893)	3,100-6,380 (4,140)	4,800-7,500
Body width (BW)	475-675 (535)	320-510 (430)	220-240
Ratio of BL/BW	12.88 :1	19.63:1	-
Head collar	300-380 (336)	230-325 (248)	-
Oral sucker (OS)	100-125 (104) ×100-130 (114)	65-90 (75) ×65-90 (77)	68-120 ×70-120
Pharynx	100-130 (117) ×80-115 (96)	83-113 (94) ×63-108 (84)	80 50
Esophagus	150-210 (178)	100-200 (149)	250-295
Ventral sucker (VS)	290-340 (312) ×305-350 (326)	180-320 (225) ×200-290 (242)	630-680 ×610-680
Ratio of OS/VS	1:2.93	1:3.07	-
Ovary	75-165 (136) ×80-240 (158)	83-125 (98) ×95-200 (118)	160-175 in diameter
Anterior testis	330-640 (503) ×160-350 (245)	250-500 (344) ×160-280 (214)	420-460 ×245-320
Posterior testis	395-740 (534) ×165-375 (233)	280-550 (350) ×110-290 (206)	460-520 ×270-320
Eggs	93-108 (100) ×55-63 (60)	108-113 (109) ×65-80 (71)	92-95 ×58-60

^aTotal 10 worms recovered in the intestine of chicks at day 14 after infection.

^bTen worms (15 day-old) from an experimental chick.

^cTen *H. kusanagi* worms (25 day-old) from an experimental herring gull, *Larus crassirostris*.

Morphology of *Acanthoparyphium tyosenense* metacercariae and adults

Metacercariae (Fig. 3A): Cyst oval to round, 350-385 (377) in diameter, with thin cyst wall, a coiled larva and extensively distributed excretory granules. Two suckers, oral and ventral sucker, plainly visible. Head collar reniform, with 23 collar spines in a single row, without ventral corner spines.

Adults (Fig. 3B): Body elongate, medium, maximum width in acetabula level. Head crown distinct, bearing about 23 collar spines in a single row, without end group ones (Fig. 3C). Oral sucker small, subterminal. Prepharynx absent. Pharynx subglobular. Esophagus short. Ventral sucker large, well developed. Sucker ratio (oral sucker:ventral sucker) approximately 1:2.93. Cirrus sac well developed and contained saccular seminal vesicle. Ovary spherical, on the dextro-median line of the body. Two testes spherical, tandem. Vitellaria follicular, distributing laterally from the level of anterior testis to posterior end. Eggs operculate, golden yellow. Dimensions of each organ are shown in detail in Table 6.

Morphology of *Parvatrema* spp. metacercariae collected in *M. veneriformis* from Taean-gun

The metacercariae of *Parvatrema* spp. collected in *M. veneriformis* from Taean-gun, Chungcheongnam-do were morphologically divided into 3 types (Fig. 4). Generally, their body very tiny and ovoid, with fine tegumental spines, maximum width at acetabular level. Oral sucker large and well developed, with lateral projections on each side. Prepharynx absent. Pharynx round and muscular. Ceca inflated oval sacs, extended to the level of the ventral sucker. Ventral sucker round, posterior to midline. Sucker ratio was 2.49:1 in average. Ovary oval to round and slightly smaller than testes, dextroanterior to the right testis. Testes round, nearly symmetrical, posterolateral to the ventral sucker. Genital pore, a wide slit-like opening, in the middle between the oral and ventral suckers. Excretory bladder V-shaped with 2 arms extending to the oral sucker level. Dimensions of each organ by the morphological type are designated in detail in Table 7.



Fig. 2. *Himasthla alincia*. (A) Metacercaria detected in a *Mactra veneriformis* clam from Taean-gun, Chungcheongnam-do (Scale bar = 75 μ m). (B) Adult (Semichon's acetocarmine stained) recovered from the small intestine of a chick experimentally infected with metacercariae (Scale bar = 1,000 μ m). (C) The head collar of an adult with 31 collar spines that included 4 lateral end-group ones (encircled) on each side (Scale bar = 100 μ m).

Table 6. Comparison of the dimensions of *Acanthoparyphium tyosenense* adults with those of previous studies

Organs	Present study (2017) ^a Range (average)	Chai et al. [13] ^b Range (average)	Kim [12] ^c Range
Body length (BL)	2,275-2,700 (2,450)	2,480-3,130 (2,830)	2,900-3,500
Body width (BW)	700-775 (725)	520-730 (630)	600-680
Ratio of BL/BW	3.38 :1	4.49:1	-
Head collar	305-320 (312)	250-300 (270)	260-290
Oral sucker (OS)	90-105 (97) x 125-135 (127)	90-110 (100) in diameter	100 diameter
Pharynx	95-100 (99) x 55-65 (59)	80-100 (90) x 60-90 (70)	87-95 x 37-40
Esophagus	200-240 (216)	170-280 (220)	250-420
Ventral sucker (VS)	290-340 (307) x 330-365 (349)	240-320 (290) in diameterx	270-300 360-380
Ratio of OS/VS	1:2.93	1:2.90	-
Ovary	100-125 (117) x 135-170 (155)	110-140 (120) x 130-180 (150)	90-100 x 120-160
Anterior testis	215-325 (278) x 350-435 (396)	260-330 (290) x 310-390 (350)	240-370 x 400-460
Posterior testis	275-375 (330) x 350-415 (369)	320-370 (340) x 290-380 (340)	230-340 x 320-450
Eggs	93-98 (95) x 54-59 (56)	105-111 (108) x 55-63 (59)	80-110 x 60-65

^aTotal 10 worms recovered in the intestine of chicks at day 14 after infection.

^bTen worms (15 day-old) from an experimental chick.

^cTen worms (15 day-old) from an experimental herring gull, *Larus crassirostris*.

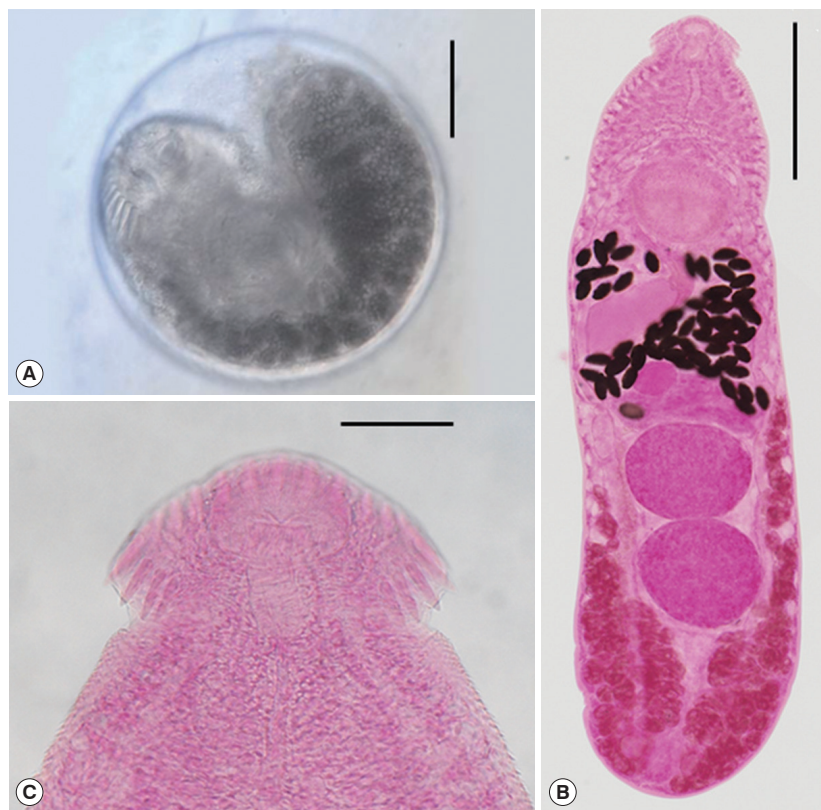


Fig. 3. *Acanthoparyphium tyosenense*. (A) Metacercaria detected in a *M. veneriformis* clam from Gochang-gun, Jeollabuk-do (Scale bar = 100 µm). (B) Adult (Semichon's acetocarmine stained) recovered from the small intestine of a chick experimentally infected with metacercariae (Scale bar = 500 µm). (C) The head collar of an adult with 23 collar spines (Scale bar = 100 µm).

Table 7. Dimensions^a of *Parvatrema* sp. metacercariae collected in estuarine clams, *Mactra veneriformis*, from Taean-gun

Organs	Type A Range (average)	Type B Range (average)	Type C Range (average)
Body length (BL)	225-288 (250)	195-230 (211)	178-223 (199)
Body width (BW)	130-150 (142)	128-148 (138)	140-150 (147)
Ratio of BL/BW	1.76:1	1.53:1	1.35:1
Oral sucker (OS)	58-65 (61) x 65-75 (69)	55-65 (61) x 60-70 (66)	50-65 (57) x 63-73 (67)
Ratio of OS/BL	1:3.85	1:3.32	1:3.21
Pharynx	23-30 (27) x 18-25 (22)	18-23 (20) x 15-23 (18)	18-33 (25) x 18-33 (23)
Ventral sucker (VS)	23-28 (25) x 28-30 (28)	23-25 (24) x 25-30 (28)	20-28 (24) x 25-30 (27)
Ratio of OS/VS	2.60:1	2.44:1	2.43:1
Ovary	18-30 (23) x 13-23 (18)	18-28 (23) x 13-23 (18)	18-25 (23) x 13-23 (18)
Right testis	25-40 (31) x 15-30 (24)	23-30 (28) x 20-25 (22)	23-33 (27) x 18-25 (21)
Left testis	25-33 (29) x 20-28 (23)	25-30 (28) x 18-25 (22)	23-30 (26) x 18-25 (22)
Vitellaria	18-30 (24) x 15-25 (17)	15-20 (17) x 10-15 (14)	18-23 (19) x 10-15 (14)

^aTen metacercariae each were measured (unit = μm).

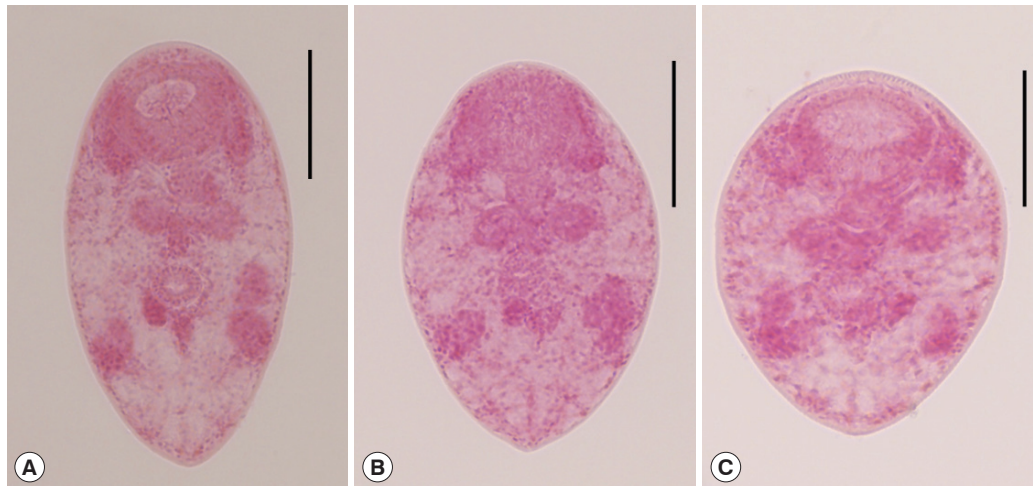


Fig. 4. The metacercariae of *Parvatrema* sp. (Semichon's acetocarmine stained) detected in *M. veneriformis* clams from Taean-gun, Chungcheongnam-do. (A) Type A is morphologically similar to *P. chaili* metacercariae. (B) Type B is apparently similar to *P. duboisi* metacercariae, although their body size is much smaller than those of *P. duboisi*. (C) Type C is more or less plump in shape (Scale bars = 75 μm).

DISCUSSION

By the present study, it has been clarified that more than 3 species of trematode metacercariae, i.e., *H. alincia*, *A. tyosenense*, and *Parvatrema* spp., are prevalent in 3 species of clams (*M. veneriformis*, *R. philippinarum*, and *C. sinensis*) from 3 west coastal regions, i.e., Taean-gun, Buan-gun, and Gochang-gun. *G. seoi* metacercariae were still prevalent in oysters from Shinan-gun, Jeollanam-do. Among more than 4 species of trema-

todes detected in this study, at least 2 (*A. tyosenense* and *G. seoi*) are human-infecting flukes [17-20]. Chai et al. [14] first reported 10 human cases of *A. tyosenense* infections, with 1-107 worm burdens, in an endemic area of heterophyid flukes in Buan-gun, Jeollabuk-do, Korea [21]. Lee et al. [17] described *Gymnophalloides seoi* n. sp. (Digenea: Gymnophallidae), which was recovered from a Korean woman suffering from acute pancreatitis and gastrointestinal troubles. This human case was the first human infection by a gymnophallid [17]. After

then, the village where the patient resided was found to be a high endemic area of *G. seoi*; additional endemic areas were also found thereafter [18-22].

Chai et al. [14] investigated 5 species of bivalves (*M. veneriformis*, *S. grandis*, *M. petechialis*, *C. sinensis*, and *S. broughtonii*) and 1 species of marine snail (*N. bicolor*) to know the infection sources of *A. tyosenense*. They detected *A. tyosenense* metacercariae in 3 molluscan species, *M. veneriformis*, *S. grandis*, and *N. bicolor*, purchased from the endemic area (Buan-gun, Jeollabuk-do). The prevalence of *A. tyosenense* metacercariae was 100% in 3 molluscan species, and the density was 134 metacercariae in *M. veneriformis*, 133 in *S. grandis*, and 23 in *N. bicolor*. Kim [13] also surveyed the metacercarial infection status in 3 species of clams, *M. veneriformis*, *C. sinensis*, and *Solon strictus*, collected from Naechodo (Island) in the estuary of Geumgang (River); total 208 *A. tyosenense* metacercariae were detected in 63 out of 100 *S. strictus* examined. In the present study, *A. tyosenense* metacercariae were found only in *M. veneriformis* collected from Taean-gun and Gochang-gun, and their prevalences (mean density/clam infected) were 50.0% (2.1) in Taean-gun and 70.0% (2.8) in Gochang-gun. Unlike Chai et al. [14], *A. tyosenense* metacercariae were not detected in 20 *M. veneriformis* collected in Buan-gun in this study. The endemicity of *A. tyosenense* metacercariae in this study was more or less similar with that of Kim [13]; however, it was very low when compared with that of Chai et al. [14].

Since Lee et al. [5] first reported that the oyster is the second intermediate host of *G. seoi*, some epidemiological studies on the infection status of oysters with metacercariae were done in coastal areas of Korea [5,7,8]. Especially, the oysters naturally produced in Aphae-myeon, Shinan-gun, Jeollanam-do [18] were most frequently investigated. Lee et al. [5] detected 2-4,792 (av. 610) metacercariae of *G. seoi* in 50 oysters from Aphae-myeon. Later, Lee et al. [7] also reported av. 786 metacercariae in 20 oysters from Aphae-myeon. Sohn et al. [8] investigated the monthly infection status with *G. seoi* metacercariae in 20 oysters each time collected in the same site of Aphae-myeon; total 248 (88.6%) oysters were infected with av. 1,339 metacercariae. In the present study, *G. seoi* metacercariae were detected in all 30 (100%) oysters from Aphae-myeon, with the mean density of 646 per oyster. Therefore, the mean metacercarial density in this study was similar with that of Lee et al. [5], slightly lower than that of Lee et al. [7], and much lower than that of Sohn et al. [8]. On the other hand, Lee et al. [5] mentioned that the metacercarial density had no relationship with the size of oysters. How-

ever, in Sohn et al. [8] and the present study, the metacercarial density was proportional to the increase of the oyster weight.

Among the 27-nominated species of the genus *Himasthla*, 3 species, namely, *H. alincia*, *H. kusasigi*, and *H. megacotyle*, were reported in Korea [12,16,23]. The morphological characteristics of *H. alincia* are highly similar to those of *H. kusasigi*, especially in that they commonly have 31 collar spines, whereas *H. megacotyle* bears 28 collar spines. However, *H. kusasigi* is distinguished from *H. alincia* in that it has a more slender body and its vitelline follicles extended more anteriorly toward the end of the cirrus sac (Table 7) [24,25]. In this respect, our specimens were identified as *H. alincia* (Fig. 1). The metacercariae of this fluke were detected in 5 species of bivalves (*M. veneriformis*, *S. grandis*, *C. sinensis*, *M. petechialis*, and *R. philippinarum*) from a coastal area in Buan-gun, Jeollabuk-do [16]. In the present study, they were detected in 3 species of bivalves (*M. veneriformis*, *C. sinensis*, and *R. philippinarum*) from 3 western coastal areas (Taean, Buan, and Gochang) of Korea. The metacercarial density was much higher in Han et al. [16] than in this study.

In the genus *Acanthoparyphium*, 14 species, namely, *A. phoenicopteri*, *A. spinulosum*, *A. ochthodromi*, *A. marilae*, *A. squatarolae*, *A. charadrii*, *A. kurogamo*, *A. melanittae*, *A. spinulosum suzugamo*, *A. tyosenense*, *A. paracharadrii*, *A. lobarichis*, *A. haematopium*, and *A. macracanthum*, have been listed so far [26-28]. Of these, *A. tyosenense* was originally described with worms from the small intestine of the duck *Melanitta fusca stejnegeri* and *M. nigra americana* caught in Korea [24]. This fluke was treated as a synonym of *A. kurogamo* by Skrjabin [29] and Chen [27]. However, *A. tyosenense* is a valid species, and it differs from *A. kurogamo* by its body shape and position of the acetabulum and testes [14,24]. Although another species, *A. marilae*, has been reported in Korea [15], the morphological characteristics of our specimens are well corresponded with those of *A. tyosenense* (Fig. 2). Comparative dimensions are designated in Table 6.

Until now, 3 species in the genus *Parvatrema* (Digenea: Gymnophallidae), i.e., *P. duboisi* (= *P. timondavidi*), *P. chaili*, and *P. homoeotectum*, have been reported in Korea [4,10,23]. Among them, *P. homoeotectum* was described only with adults, which were detected in the intestines of a migratory bird, the Mongolian plover (*Charadrius mongolus*), from the coastal area of Gunsan-si (City), Jeollabuk-do [23]. The remaining 2 species were described not only based on the adults but also on the metacercarial stage. Yu et al. [4] first detected *P. duboisi* metacercariae from intertidal clams, *R. philippinarum*, and de-

scribed with adults recovered from experimental mice. Sohn et al. [6] subsequently surveyed on the infection status with *Parvatrema* sp. metacercariae in *R. philippinarum* from 13 areas in southern coastal areas of Korea. After that time, Sohn et al. [10] erected a new species, *P. chaii* n. sp., with adults recovered from mice experimentally infected with metacercariae from surf-clams, *M. veneriformis*, which were collected from a tidal flat located in Seocheon-gun, Chungcheongnam-do, Korea. In the present study, we found *Parvatrema* spp. metacercariae in 2 species of clams, *R. philippinarum* and *M. veneriformis*. Especially, in *M. veneriformis* clams collected from Taean-gun, lots of *Parvatrema* spp. metacercariae were detected and they were morphologically divided into 3 types (Fig. 3). The metacercariae Type A were morphologically similar to *P. chaii* metacercariae although their body shape (ratio of BL/BW=1.76:1) was slightly different from that of *P. chaii* (1.98:1). The ratio of OS/VS was the same, 2.60:1, and the ratio of OS/BL was slightly different from each other (Table 7). Moreover, *P. chaii* metacercariae were previously detected in the same clam hosts, *M. veneriformis*, collected in Seocheon-gun, Chungcheongnam-do, which is located nearby in the surveyed area of this study, Taean-gun [10]. However, the remaining 2 types of metacercariae (Type B and C) were morphologically different from *P. chaii* metacercariae [10]. The metacercariae Type B was apparently similar to *P. duboisi* metacercariae; however, their body size was much smaller than that of *P. duboisi*. Further studies with molecular analysis are needed in the future to clarify the taxonomic positions of *Parvatrema* spp. in Korea.

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CONFLICT OF INTEREST

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

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