

Parvatrema timondavidi (Digenea; Gymnophallidae) transmitted by a clam, *Tapes philippinarum*, in Korea

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Abstract: Metacercariae of *Parvatrema timondavidi* (Digenea; Gymnophallidae) were found from *Tapes philippinarum*, one of the most common marine clams in Korea. *T. philippinarum* was collected from a fishery market in Seoul, and all of the clams examined were found to contain many gymnophallid metacercariae. To get adult worms, 10 ICR mice were fed with 100 metacercariae each and sacrificed by cervical dislocation at 7, 10 and 14 days after the infection. The adult worms harvested from the intestinal contents of the mice were morphologically characterized by oval body shape, large oral sucker with lateral projections on the lip, absence of the ventral pit, single cluster of vitellaria, and separately located genital pore from the ventral sucker. Based on these characters they identified as *P. timondavidi* Bartoli, 1964. This study first confirms the presence of *P. timondavidi* metacercariae in *T. philippinarum* in Korea.

Key words: *Parvatrema timondavidi*, *Tapes philippinarum*, ICR mouse

INTRODUCTION

The flukes belonging to the family Gymnophallidae are very minute intestinal parasites. Shore birds are their natural final hosts and various marine clams play the role of the first and second intermediate hosts. They are morphologically divided into six genera by some characteristic features such as the presence of the ventral pit or the location of the genital pore (Ching, 1973).

In Korea, the distribution of gymnophallid worms has been known through the adult stage of *Gymnophallus macrostoma* found from *Melanitta nigra americana* captured in Chollanam-do (Yamaguti, 1939), but other genera of family Gymnophallidae were not

discovered. Recently, however, human infection with a kind of gymnophallid fluke (*Gymnophalloides seoi* n.sp.) was found in 1988 from a patient clinically diagnosed as acute pancreatitis and cholecystitis (Lee *et al.*, 1993). Thereafter, the importance of gymnophallid worm as a human intestinal fluke was newly recognized.

In the course of the study to discover the source of human infection, we incidentally found the metacercariae of *Parvatrema* sp. from *T. philippinarum*. After infection of those metacercariae to mice, we identified the adult flukes as *P. timondavidi* Bartoli, 1964.

MATERIALS AND METHODS

1. Collection of the metacercariae

In August 1989, marine clams, *T. philippinarum* were purchased from a fishery market in Seoul. Metacercariae were collected

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from the tissue between the mantle and shell, and fixed in 10% neutral formalin. They were stained with Semichon's acetocarmine and mounted in resin.

2. Experimental infection to mice

Ten mice (ICR) were infected each with 100 metacercariae. Three to four mice were sacrificed by cervical dislocation at 7, 10 and 14 days after the infection. The small intestine was resected and opened along the mesenteric border. The intestine was stored in cold physiological saline solution at 4°C for 2 hours. The adult worms were collected from the intestinal content under a dissecting

microscope, and fixed with 10% neutral formalin under a cover slip pressure, then measured. The internal structures were observed after Semichon's acetocarmine staining.

RESULTS

1. Status of metacercarial infection in the clams

Mean number of metacercariae per clam (mean size 3.4 × 2.4 × 1.3 cm) was 14.4 (Fig. 1). All metacercariae were not encysted but covered with gelatinous substance which seemed to be originated from the clam (Fig. 2).

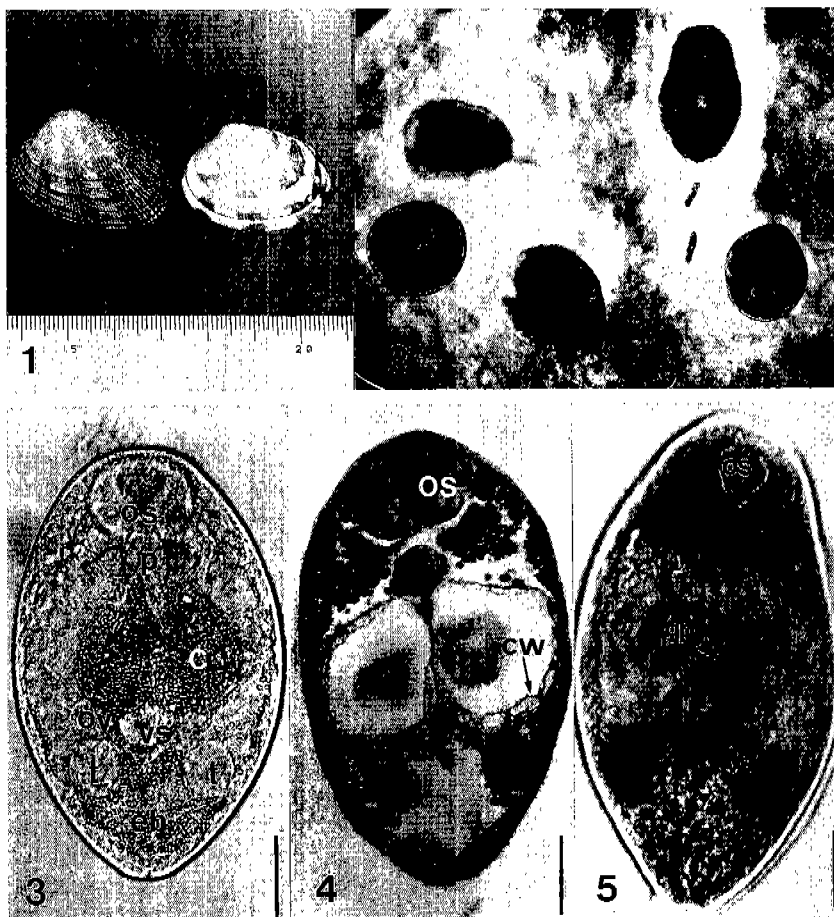


Fig. 1. *Tapes philippinarum* purchased at a fishery market in Seoul. **Fig. 2.** Unencysted metacercariae parasitized on the surface of the mantle in *T. philippinarum*. **Fig. 3.** A metacercaria of the *P. timondavidi*. c: ceca, eb: excretory bladder, os: oral sucker, ov: ovary, p: pharynx, t: testis, vs: ventral sucker. Bar: 50 μ m. **Fig. 4.** Coronal section of a metacercaria. The cecum wall was lined with one layer of large columnar cells. cw: ceca wall. Bar: 50 μ m. **Fig. 5.** A metacercaria showing a small round genital pore (gp). Bar: 50 μ m.

The metacercariae were mainly located between the mantle and shell of the clam. Even with naked eyes, they were recognized as white dots.

2. Morphology of the metacercaria

Body very tiny and ovoid, covered entirely with fine spines arranged in transverse rows. Anterior end round and posterior end a little pointed. Body length 312 μm (290-330 μm), width at acetabular level 202 μm (190-220 μm) (Fig. 3). Oral sucker large and well developed, 67 μm (63-70 μm) by 80 μm (78-83 μm). Lateral projections characteristically present on the lip. Prepharynx absent. Pharynx round and muscular, 32 μm (30-35 μm) by 27 μm (25-28 μm). Ceca inflated oval sacs, extended to the level of the ventral sucker. It's internal wall lined with one layer of large columnar cells (Fig. 4) and many granular substances filled the lumen. Ventral sucker round, 31 μm (30-33 μm) by 36 μm (33-38 μm), located posterior to midline. Sucker ratio was 2.2:1.

Testes round, nearly symmetrical, postero-lateral to the ventral sucker, 37 μm (31-41 μm) by 38 μm (33-41 μm). Ovary oval to round and slightly smaller than testes, 34 μm (27-39 μm) in diameter, dextroanterior to the right testis at the level of the ventral sucker. Genital pore, a wide slit-like opening, located in the middle between the oral and ventral suckers (Figs. 5 & 6). Excretory bladder V-shaped with two arms extending to the oral sucker level, filled with many tiny refractile granules (Fig. 3).

3. Recovery rate of the adult worms

Mean recovery rate of the adult worms was 20.1% from 10 mice infected with total 1,000 metacercariae. Chronological recovery rate was presented in Table 1.

4. Morphology of the adult worms

Body oval with round anterior and pointed posterior ends, 313 μm (300-320 μm) by 190 μm (170-220 μm) (Figs. 7 & 10). Whole body surface covered with fine tegumental spines.



Fig. 6. Sagittal section of a metacercaria showing the tube like genital atrium. c: ceca, ga: genital atrium, os: oral sucker, p: pharynx, t: testis, vs: ventral sucker. Bar: 50 μm . **Fig. 7.** A ten-day-old worm from experimentally infected mouse showing prominent lateral projections on the oral sucker. L: lateral projection, sv: seminal vesicle. Bar: 50 μm . **Fig. 8.** The lateral projections on the lip of the oral sucker. **Fig. 9.** Elliptical and operculated intrauterine eggs with very thin egg shell. Bar: 25 μm .

Table 1. Recovery rates of *Parvatrema timondavidi* from mice infected with metacercariae

Days after infection	No. mice	No. MC*	No. worms recovered	Subtotal (%)
7	3	300	73	25.0
10	3	300	111	37.0
14	4	400	15	3.7
Total	10	1,000	201	20.1

*Metacercariae

Oral sucker was so stout as to strongly attach to the bottom of the petri dish. It's diameter 78 μm (73-83 μm) by 89 μm (85-95 μm). Lateral projections present on the lip of the oral sucker (Fig. 8). Prepharynx absent. Pharynx round and muscular, 26 μm (25-28 μm) by 25 μm (20-30 μm). Ceca remarkably short, widely bifurcated, far anterior to the ventral sucker. Ventral sucker located posterior to midline, 41 μm (35-50 μm) by 42 μm (38-45 μm). Sucker ratio 1.9:1. Seminal vesicle voluminous, unipartite and located anterodextral to the ventral sucker, 67 μm (50-75 μm) by 43 μm (38-50 μm). Two testes elliptical and located posterolateral to the ventral sucker, 46 μm (37-58 μm) by 37 μm (32-39 μm). Ovary elliptical, 50 μm (38-56 μm) by 40 μm (32-46 μm), anterior to the right testis, slightly anterior to the ventral sucker. Vitelline gland, a compact round or slightly lobed mass, dorsal or left to the ventral sucker. Genital pore, a wide slit-like opening on the ventral side, some distance anterior to the ventral sucker. Seminal receptacle not observed. Excretory bladder V-shaped with two arms reach to the oral sucker. Uterus occupying the entire body in mature specimens. Egg elliptical and operculated, 28 μm (27-30 μm) by 19 μm (17-20 μm). The egg shell very thin, and embryo unformed in the uterus (Fig. 9).

DISCUSSION

Ching (1973) described 6 genera of the family Gymnophallidae; *Lacunovermis*, *Gymnophalloides*, *Paragymnophallus*, *Parvatrema*, *Metogymnophallus* and *Gymnophallus*. According to her description, the genus *Par-*

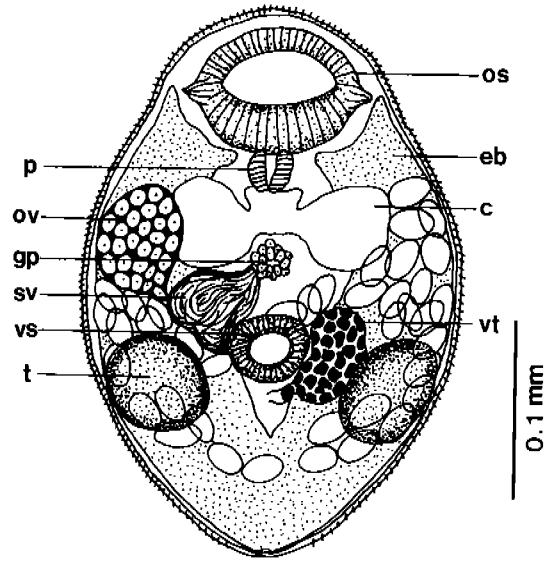


Fig. 10. Drawing of 10-day-old *P. timondavidi*. c: ceca, eb: excretory bladder, gp: genital pore, os: oral sucker, ov: ovary, p: pharynx, sv: seminal vesicle, t: testis, vs: ventral sucker, vt: vitelline gland.

vatrema has no ventral pit, but has a wide genital pore located some distance from the ventral sucker, a club-shaped unipartite seminal vesicle, lateral projections on the oral sucker, and V-shaped excretory bladder. The key to the family Gymnophallidae (Schell, 1985) additionally mentions that the genus *Parvatrema* has a single round mass of vitellaria. The present worm has the characteristic features of the genus *Parvatrema* as follows; absence of the ventral pit, wide slit-like genital pore located some distance anterior to the ventral sucker, unipartite seminal vesicle, lateral projections on the oral sucker, V-shaped excretory bladder, vitelline gland of a single cluster of follicles.

Endo (1972) redescribed the metacercaria of *P. timondavidi* Bartoli, 1964 from *T. philippinarum* caught at Tokyo Bay, with it's adult form obtained from experimental mice, which had been described as *Gymnophallus bursicola* by Ogata (1944). The present specimens obtained from experimental mice were identified as *P. timondavidi* Bartoli, 1964. Our specimens revealed some differences from the descriptions given by Endo (1972). Endo (1972)'s specimens had larger oral sucker, but

Table 2. Comparison of the measurements of *P. timondavidi* with Endo's redescription of *G. bursicola* (Ogata, 1944)(unit: μm)

	Present worm		<i>P. timondavidi</i> (Endo, 1972)		<i>G. bursicola</i> (Ogata, 1944)	
	MC	Adult	MC	Adult	MC	Adult
Body length (BL)	312(290-330)	313(300-320)	—	300	420-440	440
width	202(190-220)	190(179-220)	—	—	240-280	270
Oral sucker (OS)						
length	67(63-70)	78(73-83)	—	—	90-100	200
width	80(78-83)	89(85-95)	—	—	100-110	120
OS:BL	1:4.65	1:4.01	—	1:2.7-3.4	1:4.4-4.6	1:2.2
Pharynx length	32(30-35)	26(25-28)	—	—	28	33
width	27(25-27)	25(20-30)	—	—	40	36
Ventral sucker (VS)						
length	31(30-33)	41(35-50)	—	—	37-38	47
width	36(33-38)	42(38-45)	—	—	42-43	44
OS:VS	2.16:1	1.90:1	—	2.5-2.8:1	2.43-2.63:1	4.25:1
Testes length	38(33-43)	46(37-58)	—	—	—	64
width	35(28-41)	37(32-39)	—	—	—	42
Ovary length	—	50(38-56)	—	—	—	—
width	—	40(32-46)	—	—	—	—
Genital pore	anterior to VS		Center of ventral surface		right in front of VS	
Main distribution of uterus	entire body		hind body		posterior to VS	
Egg length	—	28(27-30)	—	—	—	22-26
width	—	19(17-20)	—	—	—	11-15
Seminal vesicle	—	unipartite	—	—	—	unipartite
Vitellaria	—	one	—	—	—	one
Excretory bladder	V-shaped	V-shaped	—	V-shaped	V-shaped	V-shaped

smaller eggs (22-26 μm) than ours (27-30 μm). The uterine tubule distribution was also different; mainly in the posterior body in the former but through the entire body in the latter. These minor differences, however, are considered as intraspecific variations. Other characters such as the location of the genital pore, presence of lateral projections of the oral sucker, the location and size of each organ, were very similar between the former and the latter (Table 2).

The natural final host of *P. timondavidi* is not known yet. The mouse seems to be one of the good laboratory final hosts of *P. timondavidi*, inferred from the high worm recovery rates and the growth status of the worms.

The present study confirmed the presence of *P. timondavidi* in Korea taking *T. philippinarum* as a second intermediate host. In case of *Gymnophalloides seoi*, another gymnophallid

fluke, it was verified that as many as 49% of residents in the endemic area were infected (Lee, 1991). Thus the possibility of human infection with other gymnophallid trematodes including *P. timondavidi* can not be ruled out at present and should be intensively studied in the future.

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= 국문초록 =

반지락 (*Tapes philippinarum*) 이 매개하는 *Parvatrema timondavidi* (Digenea; Gymnophallidae) 의 피낭유충 및 성충의 형태

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반지락 (*Tapes philippinarum*) 에서 수집한 피낭유충을 동정하기 위하여 ICR계 마우스에 피낭유충을 경구감염시킨 다음 제7일, 제10일, 제14일에 희생시키고 소장을 적출하여 충체를 회수하였다. 그 결과는 다음과 같다. 1. 피낭유충은 작은 충체 (312 × 202 μm) 가 피포하지 않은 상태로 감염되어 있고, 구흡반이 크고 잘 발달되었고 측면돌기가 관찰되었다. 장관은 이분지 되어 끝부분이 매우 부풀어 오른 맹관이었으며 복흡반까지 닿아 있었다. Ventral pit는 관찰되지 않았고 생식공은 원형으로 구흡반과 복흡반 사이에 위치하였다. 배설낭은 V자 모양으로 두팔부분은 구흡반까지 닿아 있었다. 2. 마우스 소장에서의 회수율은 평균 20.1% 이었으며 감염 후 10일 째의 회수율이 가장 높았고 14일에는 현저히 감소하였다. 3. 성충은 피낭유충과 크기의 차이가 없었다. 난황선은 한 개가 복흡반의 왼쪽 옆에 위치하였다. 저정낭은 한부분으로 이루어져 있었고 복흡반보다 전방에 위치하였다. 고환은 한쌍으로 복흡반 후측방에 위치하였고 난소는 오른쪽 고환의 전방에 위치하였다. 충란은 길이 28 μm (27-30 μm), 폭 19 μm (17-20 μm) 으로 껍질이 매우 얇았으며 난개가 있었다. 이상의 결과로 반지락을 제 2중간숙주로 하는 *Parvatrema timondavidi* (Digenea; Gymnophallidae) 가 국내에 분포함을 확인하였다.

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