

# *Parvatrema chaii* n. sp. (Digenea: Gymnophallidae) from mice experimentally infected with metacercariae collected from surf-clam, *Mactra veneriformis*

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**Abstract:** *Parvatrema chaii* n. sp. (Digenea: Gymnophallidae) is described using the worms recovered from experimentally infected mice in Korea. The metacercariae were collected from surf-clams, *Mactra veneriformis*, from a tidal flat in Sochon-gun, Chungchongnam-do. The metacercariae were elliptical (0.262 x 0.132 mm), and the genital pore had an anterior arch of 16-17 sensory papillae in scanning electron microscopic view. Adult worms were ovoid to foliate (0.275-0.303 by 0.140-0.150 mm), and their characteristic features included the presence of lateral lips, short esophagus, genital pore located some distance anterior to the ventral sucker, club-shaped seminal vesicle, a compact to slightly lobed vitellarium, elliptical eggs (0.018-0.020 by 0.010-0.013 mm), and absence of the ventral pit. This gymnophallid is classified as a member of the genus *Parvatrema* because of the location of the wide genital pore some distance from the ventral sucker, and the absence of the ventral pit. It differs from previously reported *Parvatrema* species, including the type species, *P. borinquenae*. In particular, the morphologies of the vitellarium and the genital pore with an anterior arch of 16-17 sensory papillae are unique features. Therefore, we propose it as a new species, *Parvatrema chaii* n. sp. (Digenea: Gymnophallidae).

**Key words:** *Parvatrema chaii* n. sp., gymnophallid, mouse, surf-clam, *Mactra veneriformis*

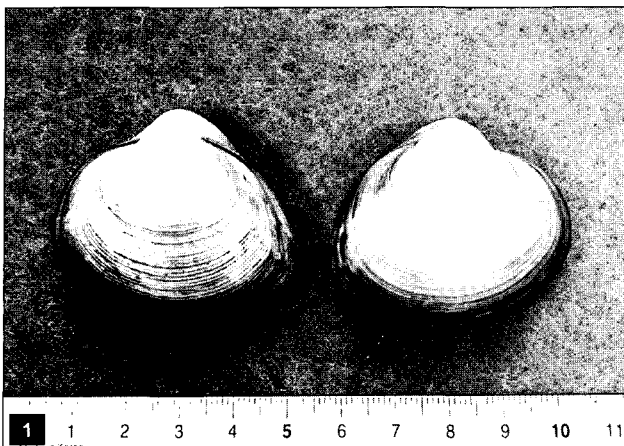
## INTRODUCTION

Gymnophallid flukes are exclusively marine parasites which commonly utilize bivalve mollusks and rarely utilize gastropods and polychaetes as intermediate hosts. Shore birds play the role of the definitive host with the exception of *Gymnophalloides seoi* (Lee et al., 1993; Ching, 1995). The metacercariae of

*Parvatrema timondavidi* were found in *Tapes philippinarum*, a marine clam species (Yu et al., 1993), and those of *Gymnophalloides seoi*, the only human-infecting species so far known, were detected in oysters, *Crassostrea gigas*, in Korea (Lee et al., 1995). In the present study, we detected gymnophallid metacercariae in surf-clams, *Mactra veneriformis*, and recovered adult worms from experimental mice that had been infected with these metacercariae. On the basis of the unique morphological characteristics of both stages, as observed via light microscopy and scanning electron microscopy (SEM), we intend to propose it as a new

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**Fig. 1.** *Mactra veneriformis*, the second intermediate host of *Parvatrema chaii*, collected from a tidal flat in Sochon-gun, Chungchongnam-do, Korea.

species, *Parvatrema chaii* n. sp. (Digenea: Gymnophallidae).

## MATERIALS AND METHODS

A total of 95 surf-clams, *M. veneriformis* (Fig. 1), were collected from a tidal flat located in Sochon-gun, Chungchongnam-do, Republic of Korea. The clams

were individually examined via pepsin-HCl digestion in order to determine the infection status of the gymnophallid metacercariae. In order to acquire adult worms, 4 mice (ICR strain) were orally infected with 300 metacercariae each, via a gavage needle. The mice were sacrificed by cervical dislocation 1 week after infection. Their small intestines were resected and divided into 3 equal pieces, then opened longitudinally. The adult worms were then harvested from the intestinal contents of each of the intestinal pieces under a stereomicroscope.

The metacercariae and adult worms were fixed using 10% neutral buffered formalin under a coverslip pressure, stained with Semichon's acetocarmine, and observed under a light microscope. Some of the metacercariae were prepared for SEM, and were observed with a SEM (ISI-Korea DS-130C) at an acceleration voltage of 10 kV.

## RESULTS

### Infection status of the clam with the metacercariae

Out of 95 clams, 94 (98.9%) were infected with

**Table 1.** Infection status of *Parvatrema chaii* metacercariae in *Mactra veneriformis*

Weight of clam (g)	No. of clams exam.	No. (%) clams posit.	No. of metacercariae detected		
			Total	Range	Average $\pm$ SD
Below 10	10	10 (100.0)	513	6 - 182	51 $\pm$ 60
10.1 ~ 15.0	22	21 (95.5)	2,546	11 - 404	121 $\pm$ 114
15.1 ~ 20.0	38	38 (100.0)	8,630	6 - 1,737	227 $\pm$ 313
Over 20.1	25	25 (100.0)	8,743	13 - 1,203	350 $\pm$ 315
Total	95	94 (98.9)	20,432	6 - 1,737	217 $\pm$ 284

**Table 2.** Recovery of *Parvatrema chaii* adults from experimental mice at week 1 after infection

Mouse no.	No. of metacercariae infected	No. of worms recovered from			
		AP <sup>a)</sup>	MP <sup>b)</sup>	PP <sup>c)</sup>	Total (%)
1	300	52	3	1	56 (18.7)
2	300	17	55	8	80 (26.7)
3	300	3	61	1	65 (21.7)
4	300	88	12	1	101 (33.7)
Total	1,200	160	131	11	302 (25.2)

<sup>a)</sup>Anterior 1/3 part; <sup>b)</sup>middle 1/3 part; <sup>c)</sup>posterior 1/3 part of the small intestine.

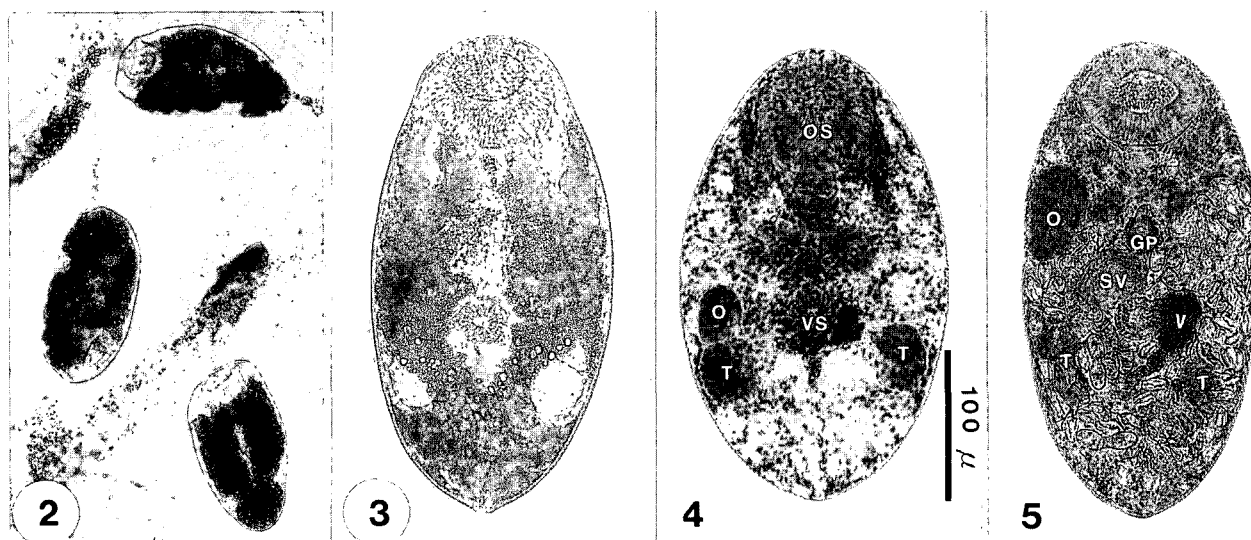


Fig. 2. Three metacercariae parasitized on the mantle of the surf-clam, *Mactra veneriformis*.

Fig. 3. A fresh metacercaria isolated from a surf-clam, not encysted and having a Y-shaped excretory bladder.

Fig. 4. A metacercaria stained with Semichon's acetocarmine. Large oral sucker (OS), smaller ventral sucker (VS), elliptical ovary (O) and 2 round testes (T) can be distinctly observed.

Fig. 5. An adult recovered from an experimental mouse 1 week post-infection (PI), and stained with Semichon's acetocarmine. Genital organs, i.e. ovary (O), genital pore (GP), seminal vesicle (SV), vitellarium (V), and 2 testes (T) can be clearly observed.

gymnophallid metacercariae (av. 217; 6-1,737 in range). The metacercarial infection status is shown in Table 1.

#### Recovery of adult flukes in experimental mice

A total of 302 adult flukes (25.2%) were recovered from 4 mice infected with 300 metacercariae each 1 week prior to the worm recovery. The recovery rate of worms from portions of the small intestine is depicted in Table 2.

### DESCRIPTION

#### *Parvatrema chaii* n. sp.

(Figs. 2-8)

**Metacercariae:** Metacercariae were collected from the tissue between the mantle and shell (Fig. 2), and were elliptical, rounded anteriorly, slightly pointed posteriorly, 233-300 ( $262 \pm 23.0$ )  $\mu\text{m}$  long, 125-140 ( $132 \pm 5.0$ )  $\mu\text{m}$  wide at midbody. Oral sucker subterminal, large and muscular, 58-68 ( $63 \pm 3.0$ )  $\times$  60-73 ( $69 \pm 3.0$ )  $\mu\text{m}$  in size, with prominent lateral projections on each side, ratio to body length 1: 3.97. Prepharynx not seen.

Pharynx well developed, muscular, 15-25 ( $20 \pm 3.0$ )  $\times$  23-25 ( $24 \pm 1.0$ )  $\mu\text{m}$  in size. Esophagus short. Ceca short, saccate, usually ending before mid-body. Ventral sucker round, 23-25 ( $25 \pm 1.0$ )  $\times$  25-28 ( $26 \pm 1.0$ )  $\mu\text{m}$  in size, located 2/5 of body length from the posterior end, sucker width ratio 1: 0.38 on average. Testes round to elliptical, 30-43 ( $34 \pm 4.0$ )  $\times$  25-30 ( $27 \pm 2.0$ )  $\mu\text{m}$  and 25-38 ( $32 \pm 3.0$ )  $\times$  23-28 ( $25 \pm 1.0$ )  $\mu\text{m}$  in size, located at both lateral sides, 1/4 body length from the posterior end. Ovary oval, 25-33 ( $29 \pm 2.0$ )  $\times$  20-25 ( $23 \pm 2.0$ )  $\mu\text{m}$  in size, located just anterior to the right testis. Vitellarium oblong, 18-28 ( $23 \pm 3.0$ )  $\times$  10-18 ( $14 \pm 2.0$ )  $\mu\text{m}$  in size. Excretory vesicle V-shaped, with anterior arms at the level of the pharynx (Figs. 3, 4).

**Adults:** Adult flukes ovoid to foliate with anterior end more rounded than the posterior end, 275-303 ( $288 \pm 10.0$ )  $\mu\text{m}$  long, 140-150 ( $147 \pm 4.0$ )  $\mu\text{m}$  wide at the midbody. Oral sucker subterminal, large and muscular, 65-73 ( $69 \pm 2.0$ )  $\times$  73-75 ( $75 \pm 1.0$ )  $\mu\text{m}$  in size, with prominent lateral projections on each side, ratio to body length 1: 4.0. Prepharynx not seen. Pharynx rounded and muscular, 20-25 ( $22 \pm 2.0$ )  $\times$  23-25 ( $23 \pm 1.0$ )  $\mu\text{m}$  in size, leading to short esophagus. Ceca short

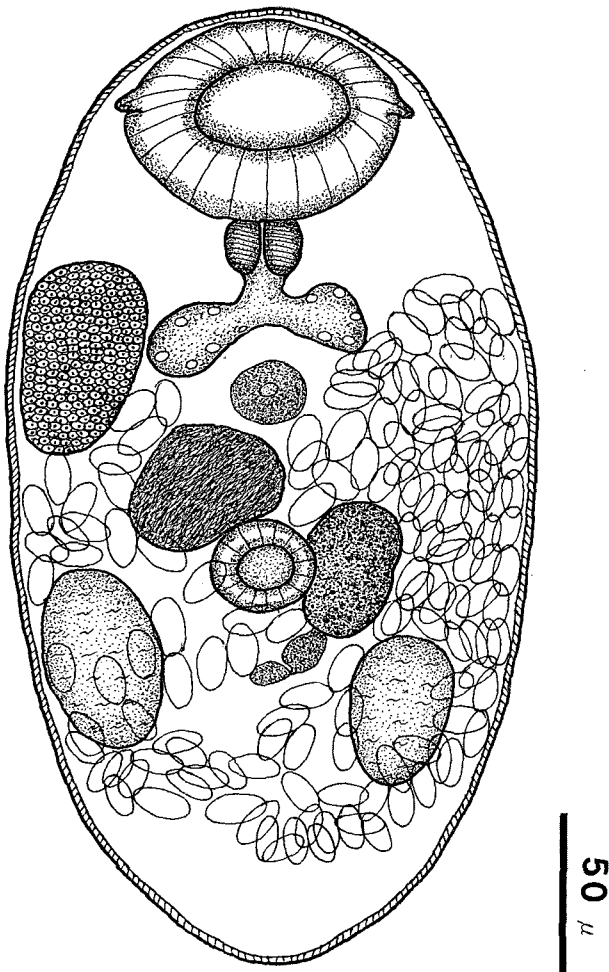


Fig. 6. Drawing of *Parvatrema chaii* n. sp. (holotype), ventral view, recovered from an experimental mouse.

and saccate. Ventral pit absent. Genital pore located some distance anterior to the ventral sucker. Ventral sucker round, 25-28 ( $26 \pm 1.0$ )  $\times$  25-26 ( $26 \pm 1.0$ )  $\mu\text{m}$  in size, located in posterior 2/5 of body length from the posterior end, sucker width ratio 1: 0.35. Testes ovoid, 43-53 ( $48 \pm 4.0$ )  $\times$  28-33 ( $30 \pm 1.0$ )  $\mu\text{m}$  and 40-50 ( $47 \pm 4.0$ )  $\times$  23-33 ( $29 \pm 4.0$ )  $\mu\text{m}$  in size, located somewhat diagonally to each other at the level of the mid hind-body. Seminal vesicle club-shaped, 30-40 ( $35 \pm 3.0$ )  $\times$  25-38 ( $30 \pm 4.0$ )  $\mu\text{m}$  in size. Ovary ovoid, 38-58 ( $50 \pm 6.0$ )  $\times$  25-40 ( $32 \pm 4.0$ )  $\mu\text{m}$  in size, located far anteriorly at the level of ceca. Vitellarium oblong, compact to slightly lobed, 30-50 ( $42 \pm 5.0$ )  $\times$  23-28 ( $25 \pm 1.0$ )  $\mu\text{m}$  in size. Uterus extending into forebody at the level of the pharynx. Eggs numerous, 18-20 ( $19.5$ )  $\times$  10-13 ( $11$ )  $\mu\text{m}$  in size. Excretory vesicle V-shaped, with anterior arms

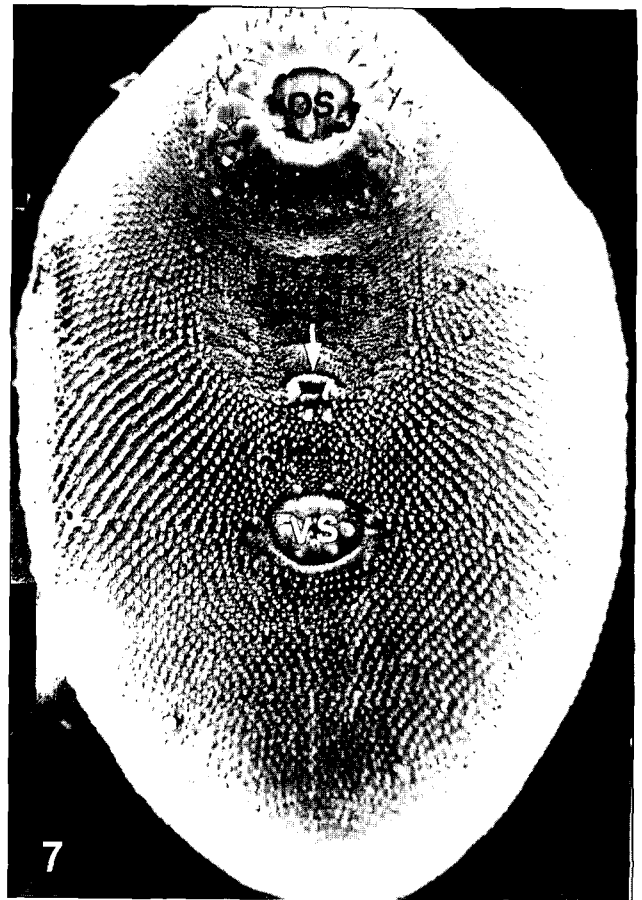


Fig. 7. Scanning electron microscopic (SEM) view of *P. chaii* metacercariae. Large oral sucker (OS), genital pore (arrow) and small ventral sucker (VS) are characteristic.

at the level of the pharynx (Figs. 5, 6).

#### Taxonomic summary

Type host: *Macra veneriformis* (intermediate) & ICR mouse (definitive).

Location: Small intestine.

Locality: Sochon-gun, Chungchongnam-do, Republic of Korea.

Specimens deposited: Holotype, GNU (Gyeongsang National University) Helm. Coll. no. 9701; paratypes, USNM Helm Coll. (Beltsville) no. 097225 & 097226.

Etymology: The specific name is in honor of Professor Jong-Yil Chai, Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine, who has dedicated all his passion to the study of parasitology in Korea.

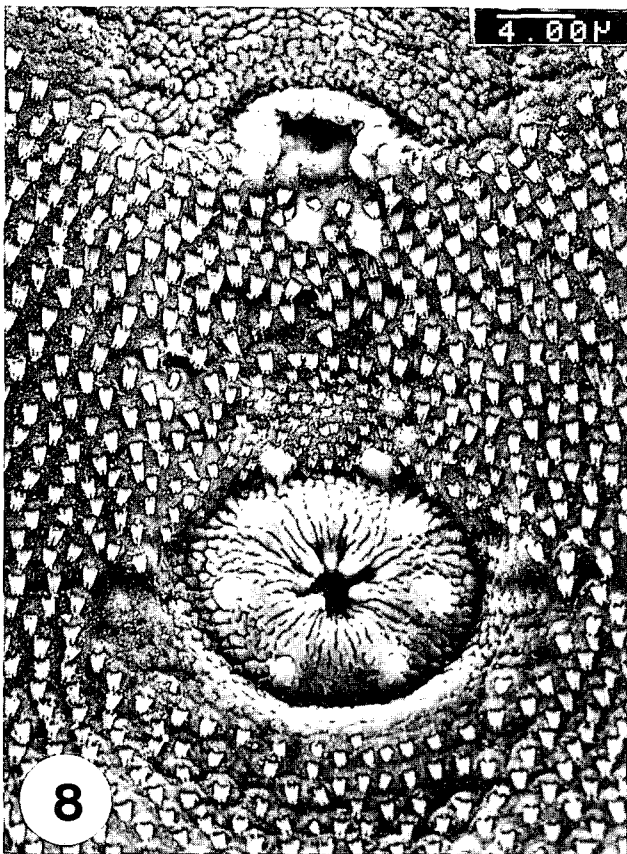


Fig. 8. SEM view of the genital pore and ventral sucker in the ventro-median portion of *P. chaii* metacercariae. The genital pore uniquely has an anterior arch of 16-17 sensory papillae.

#### SEM findings of *P. chaii* metacercariae

Metacercariae were ovoid and slightly curved ventrally. The oral sucker was approximately 2.5 times larger than the ventral sucker (Fig. 7). The genital pore was located some distance anterior to the ventral sucker and had an anterior arch of 16-17 sensory papillae. The ventral sucker was elliptical, had a radially wrinkled tegument and 6 type I papillae symmetrically distributed on its rim (Fig. 8).

#### DISCUSSION

The new trematode has been assigned as a member of the genus *Parvatrema* in consideration of the location of the wide genital pore some distance from the ventral sucker, as well as the absence of a ventral pit. It appears to have a unique arrangement of sensory

papillae surrounding the genital pore. This characteristic is difficult to see via light microscopy, but can be distinctively seen via SEM (Pekkarinen and Ching, 1994; Yu et al., 1994).

Three species previously described to have a single vitellarium, namely *P. timondavidii*, *P. borinquenae*, and *P. bushi*, are comparable with the new species. However, *P. timondavidii* has an ovoid body, an oral sucker only twice as large as the ventral sucker, and a highly lobed-vitellarium. Their egg sizes are larger than those of *P. chaii* (Yu et al., 1993). The type species, *P. borinquenae*, has been reported as a metacercaria in the style sac of a gastropod mollusk, and as an adult in charadriiform birds (Cable, 1953). The egg sizes and pharyngeal length are larger than those observed in *P. chaii* (Cable, 1953). The new species also differs from *P. bushi* in its body shape, the possession of a wide genital pore with no sensory papillae, and the shape of the vitellarium, which is compact and rounded (Ching, 1995).

The location of the ovary in the far right of the forebody in the new species is similar to that of *Meiogymnophallus macrostoma*, which was reported in shore birds in Korea. However, *M. macrostoma* differs from the new species with regard to the presence of very large paired vitellaria, and the location of the genital pore almost on the ventral sucker (Yamaguti, 1939). The new species is also different from all other previously reported *Parvatrema* species. Therefore, we propose it as a new species, *Parvatrema chaii* n. sp. (Digenea: Gymnophallidae).

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