The Korean Journal of Parasitology
Vol. 33, No. 1, 1-7, March 1995

Oysters, *Crassostrea gigas*, as the second intermediate host of *Gymnophalloides seoi* (Gymnophallidae)

Soon-Hyung LEE*, Min-Ho CHOI, Min SEO and Jong-Yil CHAI

Department of Parasitology and Institute of Endemic Diseases, Seoul National University College of Medicine, Seoul 110-799, Korea

Abstract: *Gymnophalloides seoi* has drawn medical attentions since the discovery of the first human case and a highly endemic area on a southwestern coastal island of Shinan-gun, Korea. Marine bivalves especially oysters were strongly suspected as the source of infection. In this study the oysters, *Crassostrea gigas*, naturally produced from the endemic area were examined whether they contain gymnophallid metacercariae. All of 50 oysters examined were infected with the metacercariae of a gymnophallid, with the metacercarial density per oyster of 610 on average (2-4,792 in range). Later they were identified as *G. seoi* by obtaining adult worms from experimental mice. The metacercariae were unencysted, and firmly attached on the mantle surface of the oysters with their oral sucker. In sectioned specimens they were equipped with the ventral pit, a peculiar organ of the genus *Gymnophalloides*, and non-muscular genital pore which was connected dorsally to the seminal vesicle. The seminal vesicle was in a great majority mono-sac. By this study, it has been confirmed that the oyster is a 2nd intermediate host of *G. seoi* as well as the major source of human infection with this fluke.

Key words: *Gymnophalloides seoi*, Gymnophallidae, human intestinal fluke, oysters, metacercariae, Shinan-gun, Korea

INTRODUCTION

Gymnophalloides seoi Lee, Chai and Hong, 1993 (Digenea: Gymnophallidae) was described as a new human intestinal trematode from a woman patient who suffered from acute pancreatitis, with the adult flukes collected after anthelmintic treatment and purgation (Lee *et al.*, 1993). Thereafter, its epidemiologic significance has grown remarkably, and a highly endemic area was discovered on a southwestern coastal island of Shinan-gun, Chollanam-do (Lee *et al.*, 1994). The oyster was strongly suspected as the source of infection since it has been the food of favour by most of the villagers of the endemic area (Lee *et al.*, 1994), and as the metacercariae of *Gymnophalloides tokiensis*, another species of the same genus, were described from the Japanese oyster (Ching, 1972).

In the present study we found that the oysters naturally produced nearby the endemic village were carrying the metacercariae of a gymnophallid, and identified them as *G. seoi* by obtaining adult flukes after experimental infection to ICR mice.

MATERIALS AND METHODS

More than fifty oysters, *Crassostrea gigas*

• Received Feb. 9 1995, accepted after revision Feb. 27 1995.

• This study was supported in part by a Research Grant from Seoul National University College of Medicine (1992).

* Corresponding author

(Fig. 1), were collected from Aphae-myon, Shinan-gun, the known endemic area of *G. seoi* (Lee *et al.*, 1994). The oysters were easily collected from the tideland during the ebb tide. After opening the shell with a dull knife, the mantle surface of the oysters were carefully examined under stereomicroscopy whether they contain gymnophallid metacercariae which are usually unencysted (Ching, 1965, 1972 & 1973). In order to isolate the metacercariae from the mantle, the oysters were slightly digested in artificial digestive juice at 37°C for 5 minutes. The metacercariae freed were collected under a stereomicroscope. They were washed in saline, fixed in 10% formalin, and stained with Semichon's acetocarmine, followed by mounting in canada balsam.

Some pieces of the infected oysters were fixed in 10% formalin, paraffin-sectioned, and stained with hematoxylin and eosin to observe the internal structures. Some other pieces of the infected oysters were fixed in cold 5% glutaraldehyde solution and processed for scanning electron microscopy (SEM).

In order to obtain adult flukes, five ICR mice were experimentally fed 100 metacercariae each. They were sacrificed by cervical dislocation on the 7th day after infection. The small intestine was resected and opened along the mesenteric border, and the intestinal villi were gently scratched by a glass slide to recover the worms firmly attached to the villi. The adult flukes were collected under a stereomicroscope, fixed with 10% formalin under cover slip pressure, and observed after stain with Semichon's acetocarmine. Measurements were done on 10 fixed specimens and given in micrometers (μm) with the range followed by the mean in parentheses.

RESULTS

1. Infection status of the oysters with *G. seoi* metacercariae

All of the oysters, *C. gigas* (Fig. 1), examined were found infected with the metacercariae of *G. seoi* (Table 1). The number of metacercariae infected per oyster was 610 in average, and 2-4,792 in range. The metacercarial density had no relationship with the size of oysters (data not shown). The metacercariae were mainly located on the mantle surface near the hinge of the shell, and as the infection density was increased they were spread toward the mouth part of the oyster. The heavily infected region looked as white dots even with naked eyes (Fig. 2). Peculiarly, the regions parasitized with the metacercariae showed brownish discolorations (Fig. 3).

2. Morphology of *G. seoi* metacercariae

Body small and pyriform, and covered entirely with fine tegumental spines (Fig. 4). Anterior end round and posterior end slightly pointed. The body length 346 (310-386), width 228 (205-258). Oral sucker large and well developed, 109 (94-127) by 122 (102-140), with ventrolateral lips. Pharynx round and muscular, 31 (28-37) by 37 (31-47). Esophagus very short, and succeeded to inflated oval ceca. Ventral pit located posterior to midline, 12 (7-19) by 30 (24-38). Ventral sucker located about 1/3 of body length from posterior end, 52 (46-66) by 56 (51-67). Genital pore small, inconspicuous, maximum width one half the size of ventral pit, opening at anterior margin of ventral sucker, not surrounded by prominent muscle fibers. Ovary and testes in posterior 1/3, but not easily observed due to numerous excretory granules. Excretory bladder V shaped extending to oral sucker level, filled with many tiny refractile granules (Fig. 4).

Table 1. Metacercarial density of *Gymnophalloides seoi* in oysters, *Crassostrea gigas*, collected from Aphae-myon, Shinan-gun

Metacercarial density[1)]	No. oysters
1-999	41
1,000-1,999	5
2,000-2,999	2
3,000-3,999	0
4,000-4,999	2
Total	50

[1)]No. of metacercariae/oyster: average 610 (range 2-4,792)

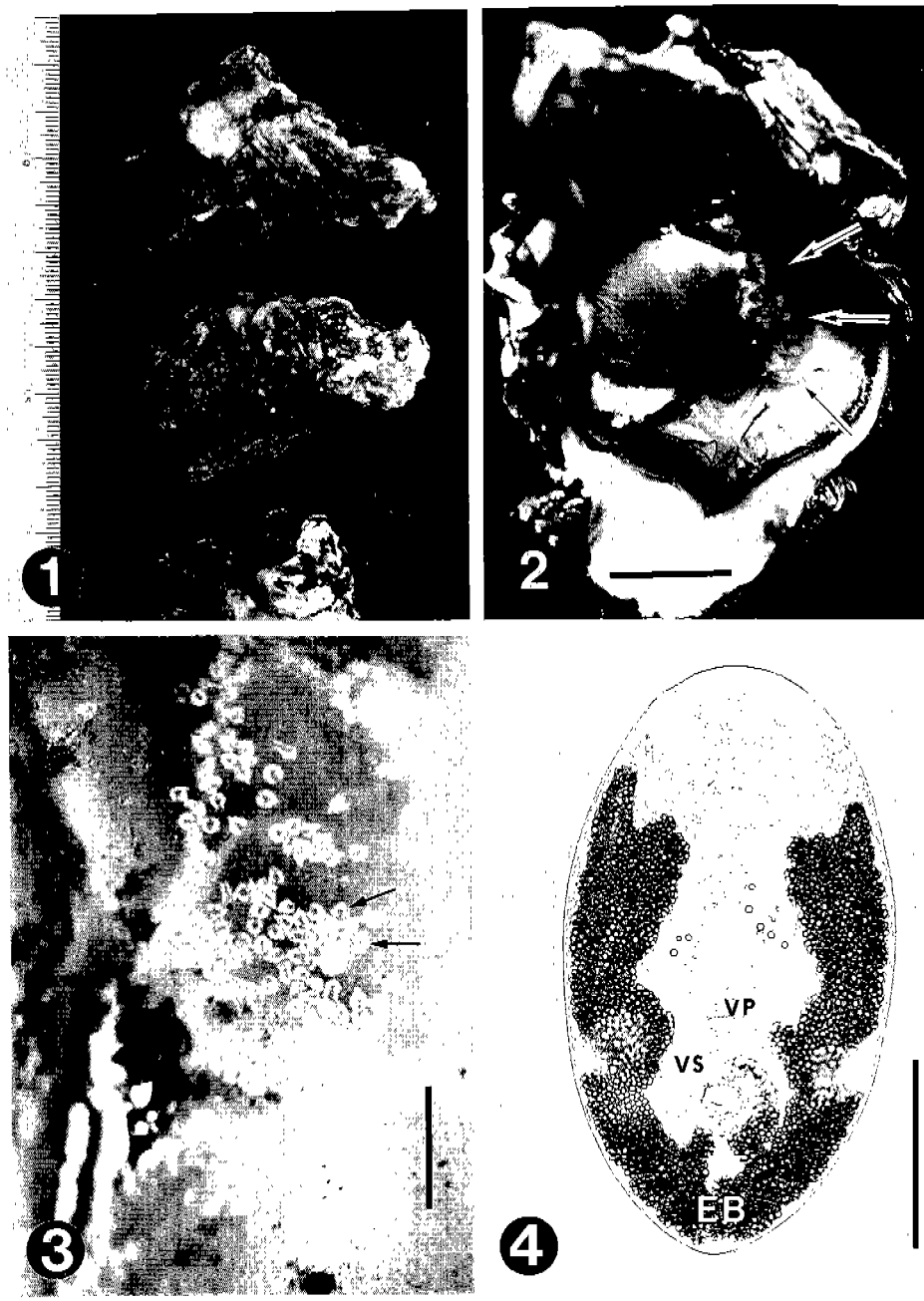


Fig. 1. The oysters, *Crassostrea gigas*, collected from Aphae Island. **Fig. 2.** Grouped metacercariae of *G. seoi* (arrows) on the mantle surface of the oyster. Scale bar: 1.5 cm. **Fig. 3.** Magnification of Fig. 2, showing numerous unencysted metacercariae of *G. seoi* (arrows) on the surface of the oyster. Scale bar: 2 mm. **Fig. 4.** A metacercaria of *G. seoi* showing the oral sucker, ventral sucker (VS), ventral pit (VP), and excretory bladder (EB). Scale bar = 100 μm.

3. Sectional and SEM views of *G. seoi* metacercariae *in situ* of the oysters

The metacercariae were sucking the extrapallial epithelia of the mantle of oysters with their well developed oral sucker (Figs. 5 & 6), just like adult intestinal flukes sucking the host's intestinal villi. They were frequently found in groups (Figs. 5 & 7). In midline sections of the metacercariae, the well developed oral sucker, short blind ceca, ventral sucker, genital pore, and ventral pit (Fig. 6) were seen. The presence of the muscular ventral pit and non-muscular genital pore at the anterior margin of the ventral sucker warranted its generic diagnosis of *Gymnophalloides* (Ching, 1972). The seminal vesicle was observed in a majority of cases as a mono-sac rather than bipartite ones.

4. Recovery and morphology of the adult fluke

The recovery rate of the adult fluke from 5 experimentally infected mice was 19.0% on average at the 7th day after the infection. The morphological details of the adults were as follows:

Body oval with round anterior and pointed posterior ends, similar to metacercariae. Length 433 (404-490), width 294 (274-315). Whole body surface covered with tegumental spines. Oral sucker so stout as to strongly attach to bottom of petri dish, 129 (117-145) by 142 (135-158). Prepharynx absent, and muscular pharynx geared to oral sucker. Esophagus short. Ceca short, saccate, and inflated, usually ending before midbody. Ventral pit median, transversely elongated, surrounded by strong muscle fibers as seen in suckers, wide, at about 1/3 of body length from posterior end. Two testes elliptical and located posterolateral to ventral sucker. Ovary elliptical, anteromedial to right testis. Seminal vesicle frequently bipartite. Genital pore small, inconspicuous, opening at level of anterior margin of ventral sucker. Vitellaria two compact follicular masses, anterolateral to ventral sucker. Uterus with anterior loops to level of pharynx, mostly in midline. Eggs embryonated, and ellipical with thin, transparent shell. Excretory bladder V-shaped, arms reaching to oral sucker.

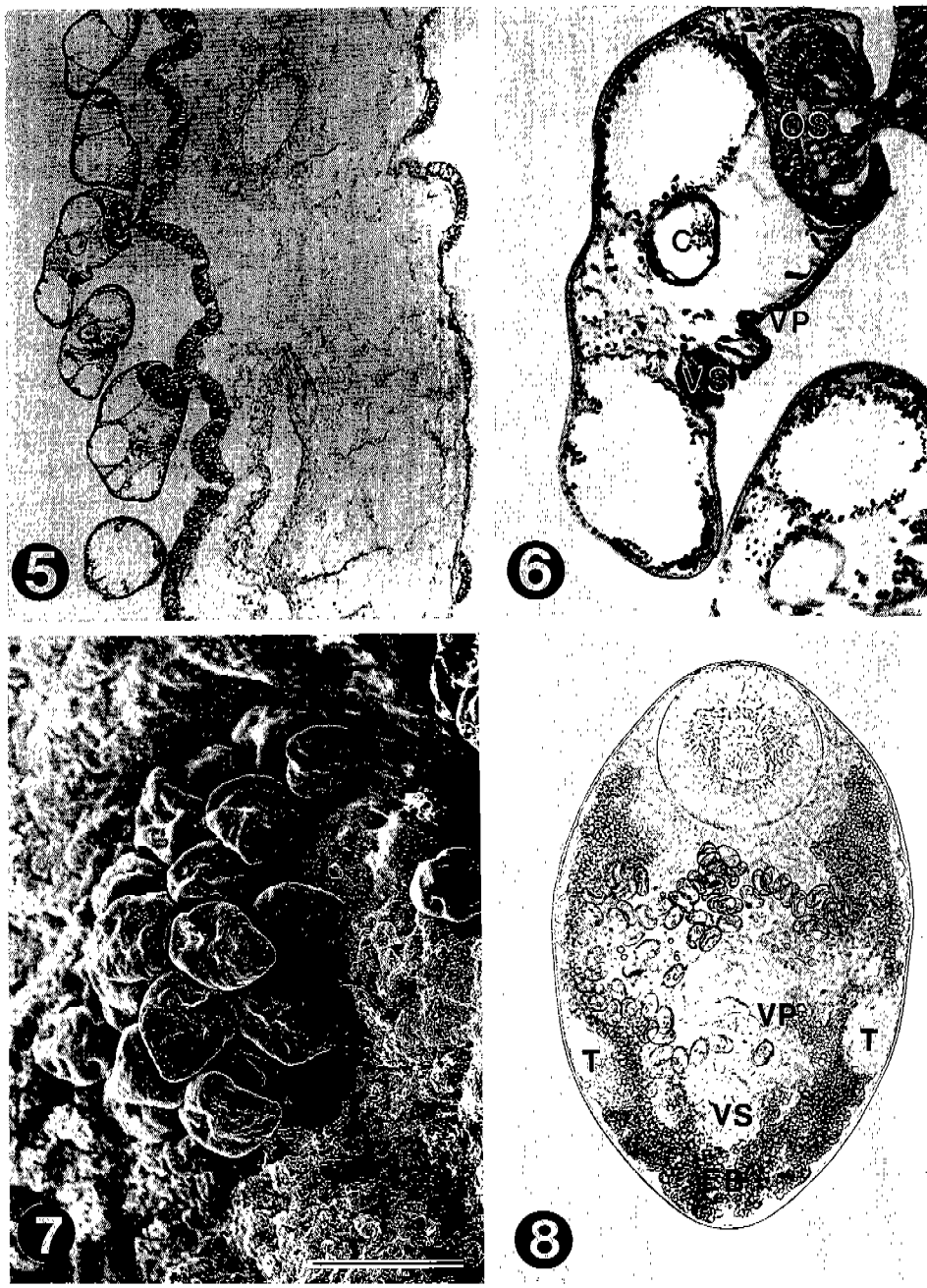
DISCUSSION

In the present study the gymnophallid metacercariae found from the oysters, *C. gigas*, collected nearby the endemic area in Shinan-gun (Lee *et al.*, 1994) were identified as those of *G. seoi*. Thus it has been confirmed that the oyster is the 2nd intermediate host and the source of human infection with *G. seoi*. The metacercarial density in infected oysters was considerably high, up to 610 on average, so that a person could be infected quite heavily by eating only one oyster raw.

Since oysters are produced not only from this area but also from other seashore areas and favoured raw by many local people in Korea, human *G. seoi* infection may not be confined to the area ever reported (Lee *et al.*, 1994), but may be more widely distributed than we suppose. Therefore, oysters produced from other localities should be examined for the presence of the metacercariae of *G. seoi*.

The metacercariae of *G. seoi* found in this study were morphologically very similar to those of *G. tokiensis*, which were originally described from oysters in Japan and redescribed by Ching (1972). Only a significant and recognizable difference in the morphology of the two species was the morphology, position, and orientation of the seminal vesicle. In *G. tokiensis* metacercariae it was bipartite and located between the ventral pit and ventral sucker, and its posterior portion was curved dorsally (Ching, 1972), whereas in *G. seoi* metacercariae it was in most cases mono-sac and situated more anteriorly between the ceca and ventral pit, and it was not curved dorsally (this study). It is known, however, that the adults of *G. seoi* frequently have bipartite seminal vesicle (Lee *et al.*, 1993). Therefore, whether these differences are only minor variations depending upon the developmental status of the metacercariae or significant features which could discriminate each species remains to be further clarified.

In spite of the morphological similarity of the metacercarial stage of *G. seoi* and *G. tokiensis*, it is taken for granted that the taxonomic validity of each species should be well



g. 5. Section of an oyster infected with *G. seoi* metacercariae. Some of them are sucking the extrapallial ıithelia of the oyster with their oral sucker. X 100. **Fig. 6.** Magnification of a metacercaria in Fig. 5, ıowing the oral sucker (OS) sucking the epithelium and tissue of the oyster, ventral sucker (VS), ventral t (VP), excretory bladder, and cecum (C). X 400. **Fig. 7.** A group of *G. seoi* metacercariae attached on e mantle surface of an oyster as observed by scanning electron microscopy. Scale bar = 179 μm. **Fig. 8.** ı adult worm of *G. seoi* recovered from the small intestine of an experimentally infected mouse. VS: ntral sucker, VP: ventral pit, T: testes, EB: excretory bladder. Scale bar = 100 μm.

reserved. Because, larval stages of different parasite species or even different genera are frequently so similar in morphology that examination of their adult stage is essential to determine the species. This strong necessity should also be applied in the cases of gymnophallid species. For example, the metacercarial stage of *Parvatrema homoeotecnum* James, 1964, *P. borinquenae* Cable, 1953, and *P. borealis* Stunkard and Uzmann, 1958 was so similar that the morphology of adult flukes and details of their life cycle were necessary for species determination (James, 1964). The adult stage of *G. seoi* is already known (Lee *et al.*, 1993) but the adult of *G. tokiensis* has not been discovered. For this reason, more detailed consideration on the taxonomic validity of the two species is not possible at present.

There are still many questions to be answered in biological and epidemiological aspects of *G. seoi*. One of the most basic problems to be solved is that we have to find out the complete life cycle of this trematode including egg hatching mechanism in water, larval development in molluscan hosts, and importance of the human host as the source of eggs and in maintaining the endemicity. Especially the first intermediate host and natural final hosts other than humans should be urgently studied.

The first intermediate host of *G. seoi* might also be the oysters (the second host), considering ever reported life cycles of other gymnophallid trematodes. In the case of *Parvatrema borealis*, sporocysts and cercariae were found in a molluscan host, *Gemma gemma*, and metacercariae in the same host and in polychaetes (Stunkard, 1962). James (1960 & 1964) described the unique life cycle of *Parvatrema homoeotecnum*, in which case the primary germinal sac, the tailed daughter sac, cercaria, and metacercaria were found in a gastropod intermediate host, *Littorina saxatilis tenebrosa*. Szidat (1962) described various developmental stages of *Gymnophallus australis* from the same host, the mussel.

ICR mice were experimentally susceptible to *G. seoi* infection in this study, although the infection did not last longer than a week (data not shown). Thus, small rodents such as house rats could take the role of a natural final host in endemic areas. However, gymnophallids are generally known as parasites of avian hosts (Yamaguti, 1971; Schell, 1985), so birds such as the oystercatcher, duck, and seagull are more suspected as natural final hosts of *G. seoi*.

REFERENCES

Ching HL (1965) Life cycles of *Lacunovermis conspicuus* n. gen., n. sp. and *Meiogymnophallus multigemmulus* n. gen., n. sp. (Gymnophallidae: Trematoda) from *Macoma inconspicua* and diving Ducks from Vancouver, Canada. *Proceed Helminthol Soc Wash* **32:** 53-63.

Ching HL (1972) A redescription of *Gymnophalloides tokiensis* Fujita, 1925 (Trematoda: Gymnophallidae). *Can J Zool* **50:** 1299-1302.

Ching HL (1973) Description of *Gymnophalloides somateriae* (Levinsen, 1881) from *Macoma inconspicua* and diving ducks from Vancouver, Canada. *Can J Zool* **51:** 801-806.

James BL (1960) A new cercaria of the subfamily Gymnophallinae (Trematoda: Digenea) developing a unique "parthenita" in *Littorina saxatilis* (Olivi). *Nature* **185:** 181-182.

James BL (1964) The life cycle of *Parvatrema homoeotecnum* sp. nov. (Trematoda: Digenea) and a review of the family Gymnophallidae Morozov, 1955. *Parasitology* **54:** 1-41.

Lee SH, Chai JY, Hong ST (1993) *Gymnophalloides seoi* n. sp. (Digenea: Gymnophallidae), the first report of human infection by a gymnophallid. *J Parasitol* **79**(5)**:** 677-680.

Lee SH, Chai JY, Lee HJ *et al.* (1994) High prevalence of *Gymnophalloides seoi* infection in a village on a southwestern island of the Republic of Korea. *Am J Trop Med Hyg* **51:** 281-285.

Schell SC (1985) Handbook of Trematodes of North America North of Mexico. University Press of Idaho, Moscow, Idaho, p. 124-125.

Stunkard HW (1962) New intermediate host for *Parvatrema borealis* Stunkard and Uzmann, 1958 (Trematoda). *J Parasitol* **48:** 157.

Szidat L (1962) Über eine Ungewohnlicke form parthenogenetischer vermehrung bei metacercarien einer *Gymnophallus*-Art aus *Mytilus platensis*, *Gymnophallus australis* n. sp. des Sudatlantik. *Z Parasitenkd* **22:** 196-213.

Yamaguti S (1971) Synopsis of Digenetic Trematodes of Vertebrates. Keigaku Publishing Co., Tokyo, p 1-1074.

=초록=

참굴큰입흡충(*Gymnophalloides seoi*)의 제2중간숙주로서 참굴의 역할

이순형, 최민호, 서 민, 채종일

서울대학교 의과대학 기생충학교실

우리 나라 고유의 인체 기생 장흡충 *Gymnophalloides seoi* Lee, Chai and Hong, 1993은 급성 췌장염 환자에서 처음 발견되었고, 신안군 도서지방에 큰 유행지가 있음이 밝혀져 의학적 중요성이 점차 높아지고 있으나, 지금까지 인체 감염원이 되는 제2중간숙주가 알려져 있지 않았다. 이번 연구에서는 자연산 참굴(*Crassostrea gigas*)이 제2중간숙주의 하나일 것으로 추정하고 신안군 유행지에서 참굴을 채집한 후 gymnophallid metacercariae에 감염되어 있는지 조사하였다. 조사한 참굴 50마리 모두에서 gymnophallid metacercariae가 발견되어 100%의 감염률을 보였고, 참굴 1마리당 평균 610개(개체당 2-4,792개)의 감염량을 보였다. 충체의 종은 추후 마우스 감염 실험을 통하여 *G. seoi*로 동정할 수 있었다. 유충(metacercariae)은 낭을 가지고 있지 않았고, 구흡반으로 참굴의 외투막(mantle surface)을 흡착하고 있었으며, 참굴의 절편 표본에서 참굴큰입흡충 속의 특징 중 하나인 복측흠(ventral pit)이 관찰되며, 복흡반 전방에 근육질이 아닌 생식공이 있고 저정낭으로 연결되어 있었다. 저정낭은 대부분의 충체에서 단낭성(mono-sac)이어서 유일한 다른 종인 *G. tokiensis*가 이낭성(bipartite) 저정낭을 가진 것과는 차이를 보였다. 이 연구를 통하여 참굴이 *G. seoi*의 제2중간숙주이며 동시에 가장 중요한 인체 감염원일 것으로 판단하였고, 이 흡충의 우리말 이름을 참굴큰입흡충으로 제안한다.

〔기생충학잡지 33(1): 1-7, 1995년 3월〕