

Redescription of *Macroorchis spinulosus* Ando, 1918 (Digenea: Nanophyetidae) encysted in the fresh water crayfish, *Cambaroides similis*

Jong-Yil CHAI^{1)*}, Woon-Mok SOHN²⁾, Sun HUH³⁾, Min-Ho CHOI¹⁾,
and Soon-Hyung LEE¹⁾

Department of Parasitology and Institute of Endemic Diseases¹⁾, Seoul National University College of Medicine, Seoul 110-799, Department of Parasitology²⁾, College of Medicine, Inje University, Pusan 614-735, Department of Parasitology³⁾, College of Medicine, Hallym University, Chunchon 220-702, Korea

Abstract: *Macroorchis spinulosus* Ando, 1918 (Digenea: Nanophyetidae) is redescribed based on adult flukes recovered from experimental mice, rats and cats which were fed with metacercariae encysted in the muscle of the crayfish, *Cambaroides similis*. A total of 117 adult flukes were harvested from the small intestine of the experimental animals at 5-21 days post-infection. The worm recovery rate was 31-37%, not significantly different among the three kinds of animals. The metacercariae were round, 0.18 mm in average diameter, encysted with two layers of thick walls, and possessing a stylet on the oral sucker and Y-shaped excretory bladder. The adult flukes were oval, 0.66-0.71 mm long and 0.36-0.41 mm wide, and characterized by the presence of a stylet on the oral sucker, variable location of the ovary near the mid-portion of the body, bilobed seminal vesicle, and anterolateral location of two testes. These morphological features were a little different from the original description, hence, an emended version of *M. spinulosus* was given. *M. spinulosus* is a new intestinal trematode of animals and possibly man in Korea.

Key words: *Macroorchis spinulosus*, Nanophyetidae, crayfish, rats, mice, cats, metacercaria, morphology, redescription

INTRODUCTION

Since the trematode genus *Macroorchis* was erected by Ando (1918) with the type species, *M. spinulosus*, 3 additional species have been reported in the literature. The one is *M. himizu* Machida and Uchida, 1982 and two others *M. chimarrogalus* Saito et al., 1982 and *M. elongatus* Saito et al., 1982. *M. spinulosus* was described from experimentally infected rats,

mice, dogs and cats in Japan, and 3 other species were found from naturally infected rats, mice, shrews or moles also in Japan. In Korea, no report was available on the presence of flukes belonging to the genus *Macroorchis*.

During the trematode survey at eastern mountainous areas of Korea, we found metacercariae belonging to the genus *Macroorchis* from the muscle of the fresh water crayfish, *Cambaroides similis*, and obtained adult flukes after experimental infection to animals. The adult flukes were identified as *Macroorchis spinulosus* Ando, 1918.

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* Corresponding author (e-mail: c jy@plaza.snu.ac.kr)

Table 1. Results of worm recovery^{a)} from three kinds of animals infected with metacercariae of *M. spinulosus* isolated from the crayfish

Kinds of animal	Days after infection	No. animals infected	Total No. mc ^{b)} given	No. worms recovered (%)
mouse	10-21	3	90	32 (35.6)
rat	5-21	7	210	78 (37.1)
cat	5-10	2	200	61 (30.5)
Total	5-21	12	500	171 (34.2)

^{a)}Most of the worms were recovered from the duodenum. ^{b)}mc; metacercariae

MATERIALS AND METHODS

The metacercariae of *M. spinulosus* were isolated from the muscle of the crayfish, *Cambaroides similis*, collected at Osongjon-ri, Hyonbuk-myon, Yangyang-gun, Kangwon-do, by artificial digestion technique. Three mice (ICR), 7 rats (Sprague-Dawley), and 2 cats were orally fed 30-100 metacercariae, and sacrificed after 5-21 days to recover flukes of variable ages. The metacercariae and adults were fixed with 10% formalin under cover slip pressure, and stained with Semichon's acetocarmine. Both fresh and stained specimens were observed under light microscopy, and 30 stained samples were measured. Several adult specimens were processed routinely for longitudinal sections, and stained with hematoxylin and eosin. Measurements were given in μm followed by the mean in parentheses, unless otherwise stated.

RESULTS

1. Recovery of worms

A total of 171 flukes, consisting of 160 adults containing eggs and 11 juveniles without eggs, were recovered at 5-21 days post-infection chiefly from the duodenum of three kinds of animals (Table 1). The overall worm recovery rate was 34.2%, and by each kind of animal, 35.6% from 3 mice, 37.1% from 7 rats, and 30.5% from 2 cats, of which the difference was statistically not significant ($p < 0.001$) (Table 1).

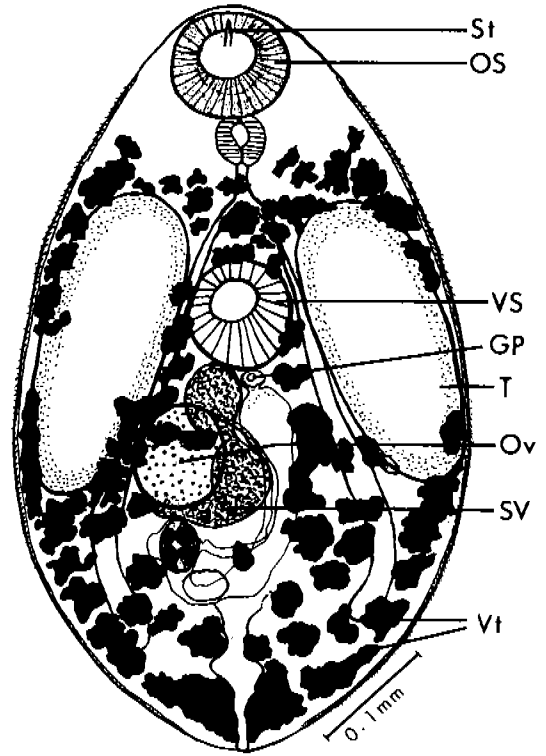


Fig. 1. *Macroorchis spinulosus*, drawing of an adult fluke recovered from an experimental rat 5 days after infection. St, stylet; OS, oral sucker, VS, ventral sucker; GP, genital pore; T, testis; Ov, ovary; SV, seminal vesicle; Vt, vitellaria.

2. Redescription of *Macroorchis spinulosus* Ando, 1918

Adults (Figs. 1-5): Body small, oval, 661-706 (684) long, and 357-414 (386) wide. Tegument of whole body armed with small scale-like spines. Oral sucker subterminal, 82-98 (90) long by 92-110 (101) wide, with a prominent

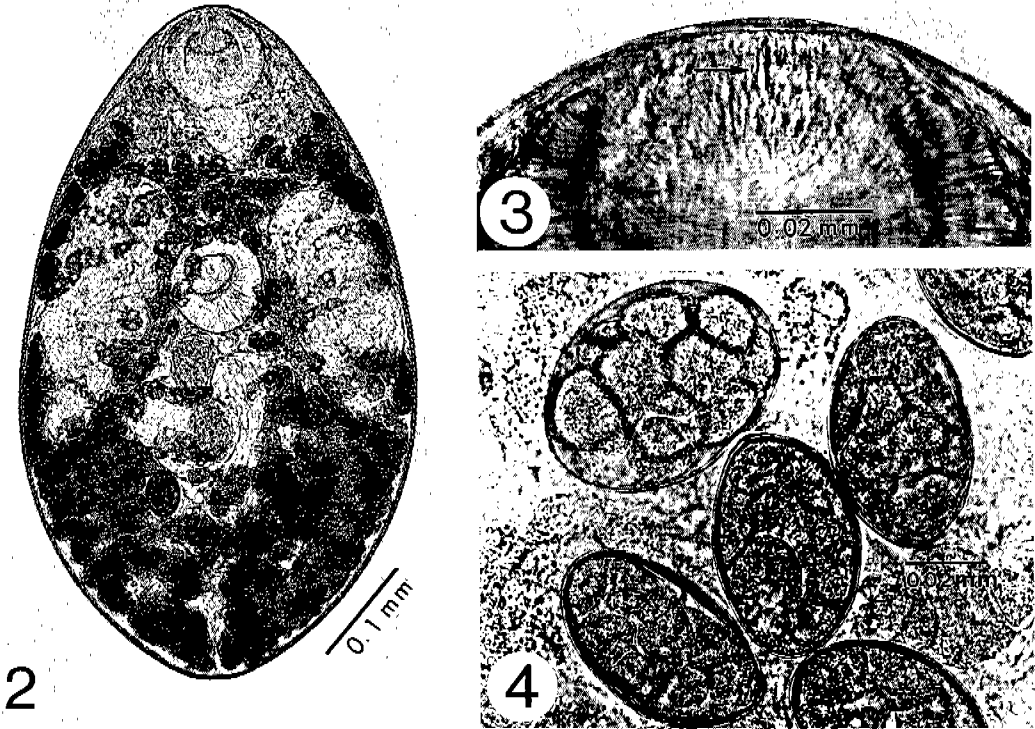


Fig. 2. The fresh specimen of *M. spinulosus* drawn in Fig. 1, ventral view. Note the bilobed seminal vesicle in the mid-portion of the body. **Fig. 3.** The oral sucker showing a stilet (arrow) on its upper lip. **Fig. 4.** Uterine eggs, immature, in a full-grown adult fluke.

Table 2. Presence of a stilet on the oral sucker and variable position of the ovary in the present specimens, *M. spinulosus*

Age of worms (days)	No. worms observed	Stilet		Ovary		
		+	-	Left	Median	Right
5	84	17	67	0	16	68
7	10	7	3	1	3	6
10-21	8	1	7	5	0	3
Total	102	25	77	6	19	77

stilet (Figs. 1-3) in about 25% of the specimens (Table 2). Prepharynx absent. Pharynx round, 40-56 (48) in diameter. Esophagus 52-54 (53) long, bifurcating a little anterior to the ventral sucker. Ceca narrow, running between the ventral sucker and testes, and terminating near 1/5-1/6 body portion from the posterior extremity. Ventral sucker round, protruded ventrally, 82-100 (91) in diameter, nearly equal in size to the oral

sucker. Testes very large, symmetrical, longitudinally elongate, and extra-cecal near the anterior part of the body. Right testis 260-293 (277) long and 98-110 (104) wide, left testis 243-274 (259) long and 93-102 (98) wide. Vas efference arising from mid-level of the testes, running transversely to enter into the anterior part of the seminal vesicle. Seminal vesicle bilobed anteroposteriorly (Fig. 1) and dorsoventrally (Fig. 5), 50-62(56) wide,

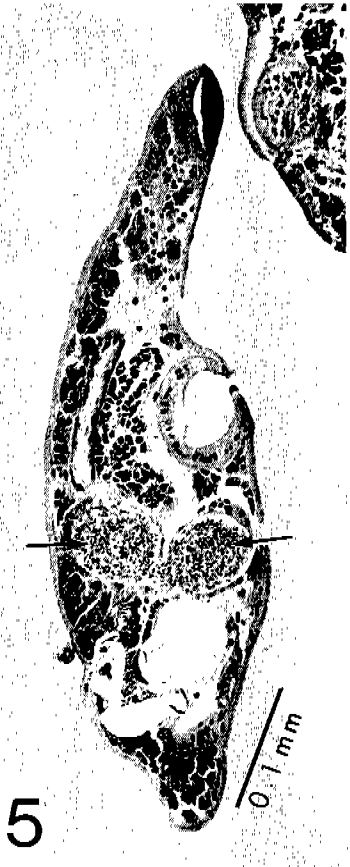


Fig. 5. A mid-sagittal section of an adult *M. spinulosus*, showing the characteristically bilobed seminal vesicle (arrows).

just posterior to the ventral sucker. Pars prostatica short, curved, connected to the ejaculatory duct and genital pore. Genital pore median, a little posterior to the ventral sucker. Ovary oval, variable in position (Table 2), median, submedian, dextral or sinistral, 74-79 (77) by 55-61 (58), a little posterodorsal to the genital pore. Oviduct arising from the anterior part of the ovary, connected to the Laurer's canal, then ascending to receive the vitelline duct. Oötype and Mehlis' gland not prominent. Vitelline follicles grouped, distributed over the greater part of the body except at the anterior part to the pharynx. Uterus postacetabular, loop-shape, descending down to the posterior extremity. Uterine eggs immature, 3-20 in number per specimen, rarely 30-40, oval or slightly elongated, 56.1-68.9 (59.6) long and 35.7-43.3 (39.6) wide (Fig. 4). Excretory

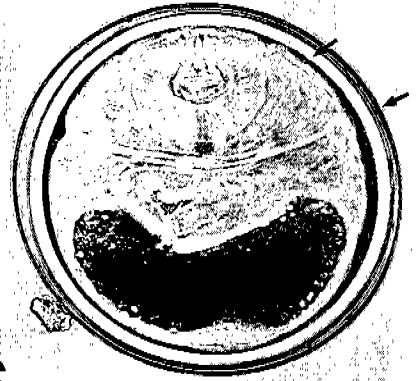


Fig. 6. A metacercariae of *M. spinulosus*, isolated from a crayfish, *C. similis*. Note a prominent stylet on the oral sucker and two thick cyst walls (arrows).

bladder Y-shaped. Excretory pore terminal.

Host: mouse, rat, cat (all experimental)

Locality: Hyonbuk-myon, Yangyang-gun, Kangwon-do, Republic of Korea

Position: upper part of the small intestine

Specimen Deposition: SNU Dept. Parasit. Helm. Coll. No. 8821-8900

Metacercariae (Fig. 6): Cyst round, 170-189 (180) in average diameter, encysted with relatively thick walls of two layers. Excretory bladder dark, large, and Y-shaped, with numerous excretory granules. Excysted metacercaria spindle shape, 400-480 (440) long and 200-270 (220) wide. Oral sucker armed with a stylet, 75-85 (79) long and 85-90 (88) wide. Acetabulum 65-70(69) in diameter. Testes extra-cecal.

Host: *Cambaroides similis*

Locality: Hyonbuk-myon, Yangyang-gun, Kangwon-do, Republic of Korea

Position: Muscle

DISCUSSION

There were some problems in the identification of the present fluke specimens as *M. spinulosus* Ando, 1918. It was largely because of insufficient and incorrect original description of *M. spinulosus*, and of the presence of *M. himizu*, a morphologically very close species to *M. spinulosus*. Another reason

was a difference in the second intermediate host, the crab (Ando, 1918) v/s crayfish (this study).

According to the original description, *M. spinulosus* has an elongated body, ovary in median position, saccular seminal vesicle, seminal receptacle located anterior to the ovary, and vitellaria extending from the level of intestinal bifurcation (Ando, 1918). These features were inconsistent with our specimens. Our specimens have in most cases an oval body, ovary in variable position, median or shifted to the right or left side, apparently bilobed seminal vesicle, absence of seminal receptacle which was also indicated by Saito *et al.* (1982), and vitellaria extending from the level of the pharynx.

Meanwhile, *M. himizu* was proposed as a new species based on differential points from *M. spinulosus* that *M. himizu* has an oval body rather than elongated one, usually larger acetabulum than the oral sucker, the oral sucker without a stylet, larger ovary and testes, anterior extent of the seminal vesicle and testes. Our specimens were similar to *M. himizu* in general morphology, but different in the presence of a stylet on the oral sucker in about 1/4 of specimens, nearly equal size of the acetabulum and oral sucker, ceca terminating near 1/5-1/6 body portion from the posterior extremity, genital pore just behind the ventral sucker, and more posterior location of the seminal vesicle.

In spite of some incompatibility we identified our specimens as *M. spinulosus* under the following considerations. First, the general shape of trematodes could be quite variable depending on the status and viability of the worms at fixation, and the pressure given to fix them. Slight variation of the shape could also be an intraspecific variation. Second, the position of the ovary could be variable too, shifted to the right or left side, if they are floating near the median area of the body, not firmly affixed to other organs. In our specimens the position of the ovary tended to change from the right and middle side at juvenile and young adult stages to the left side at the adult stage. Third, the different morphology of the seminal vesicle from our specimens and presence of a seminal

receptacle in the original description (Ando, 1918) were due to a mistake to interpret one of the bipartite seminal vesicle as a seminal receptacle (Machida and Uchida, 1982). Fourth, a slight difference in the distribution of vitellaria could be considered as a minor intraspecific variation. In all other points, our specimens were consistent with the descriptions of *M. spinulosus*.

M. spinulosus can be easily differentiated from *M. elongatus* in the smaller body size and oval shape compared with extremely large and elongated body of the latter (Saito *et al.*, 1982). The reproductive organs of *M. elongatus* are located at the anterior half of the body and the number of intrauterine eggs are more than other species including *M. spinulosus*.

M. spinulosus is differed from *M. chimarrogalus* in having a ceca ending more posteriorly, and relatively larger size of testes compared to that of the ventral sucker. The egg shell of *M. spinulosus* has an even thickness and smooth contour, while that of *M. chimarrogalus* becomes gradually thin as it approaches to the operculum and has a stumpy nodule at the abopercular side (Saito *et al.*, 1982).

In our specimens of *M. spinulosus* metacercariae, the oral sucker was a little larger than the ventral sucker. On the contrary, in the original description, they have larger ventral sucker. The difference was, however, minor, and is considered not significant.

The fresh water crayfish, *Cambaroides similis*, is a new second intermediate host of *M. spinulosus*. It is also known to play an important role as a second intermediate host of *Paragonimus westermani* (Choi, 1990). In the original paper of *M. spinulosus* (Ando, 1918), the second intermediate host was only the fresh water crab. In this study, the metacercariae were found from the muscle of the crayfish collected at an eastern mountainous area of Korea, and it was impossible to collect crabs from that area. The metacercarial burden was 10-30 per positive crayfish (data not shown). As for other species of *Macroorchis* found in Japan, the metacercariae of *M. elongatus* were found from the fresh water crab, *Potamon dehaani* (Saito

et al., 1982) and those of *M. chimarrogalus* were from aquatic insects, i. e., the naiades of stoneflies and larvae of dobsonflies (Saito et al., 1982).

House rats were reported as a natural final host of *M. spinulosus* (Ando, 1921). Experimental final hosts include dogs, cats, mice, rats, guinea pigs, and rabbits (Ando, 1918). In the present study, cats, mice, and rats were reconfirmed as susceptible laboratory hosts for *M. spinulosus*.

As to the possibility of human infection with *M. spinulosus*, there has not yet been sufficient evidence. Ando (1921) reported two egg positive human cases in stool examination, but did not collect adult flukes. He also reported successful experimental infection of humans, but his confirmation was based only on stool examination for eggs. It is well known that the crayfish, *C. similis*, is popularly eaten under improperly cooked conditions by the people in rural areas of Korea. Therefore, possibility of natural human infection with *M. spinulosus* remains to be further studied in eastern mountainous areas of Korea.

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=초록=

참가재(*Cambaroides similis*)에 피낭한 가시큰고환흡충
(*Macroorchis spinulosus* Ando, 1918)의 再記錄

채종일¹⁾, 손은목²⁾, 허 선³⁾, 최민호¹⁾, 이순형¹⁾

서울대학교 의과대학 기생충학교실 및 풍토병연구소¹⁾, 인제대학교 의과대학 기생충학교실²⁾, 한림대학교 의과대학 기생충학교실³⁾

강원도 양양군 현북면 어성전리에 서식하는 참가재(*Cambaroides similis*)의 근육에서 *Macroorchis spinulosus* Ando, 1918(Digenea: Nanophyetidae)의 피낭유충을 검출하고 마우스, 흰쥐 및 고양이에 실험감염시켜 성충을 얻은 다음 형태학적 특징을 관찰하였다. 실험동물들의 소장으로부터 감염 5-21일에 총 117마리의 충체를 회수하였으며, 평균 충체회수율은 31-37%로서 동물별로 큰 차이가 없었다. 피낭유충은 둥글고, 평균 직경 0.18 mm이며, 2개의 두꺼운 막으로 둘러싸여 있고, Y-자형의 배설낭을 가지고 있었다. 성충은 대부분 난형이며, 길이 0.66-0.71 mm, 폭 0.36-0.41 mm이었다. 구흡반에는 칩(stylet) 1개가 있고, 난소가 체중앙부 근처에서 다양하게 위치하며, 저정낭이 이낭형(bilobed)이고, 두 고환이 약간 전측방에 위치하는 점 등의 형태학적 특성을 나타내었다. 실험 감염에서 얻은 성충들을 *M. spinulosus*로 동정할 수 있었으나 그 형태가 최초 기록과 몇 가지 다른 점이 있어 앞으로 이 흡충의 원활한 진단을 위해 충체의 형태를 수정하여 재기록하였다. 이 장흡충은 국내에는 미기록종이며 포유동물을 종숙주로 하고 있을 것으로 추측되며 인체 감염 가능성도 배제할 수 없다. 이 충체의 우리말 이름을 가시큰고환흡충으로 부를 것을 제안한다.

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