

# Studies on Intestinal Trematodes in Korea

## IV. Geographical Distribution of *Pygidiopsis* and *Heterophyes* Metacercariae

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### INTRODUCTION

The family Heterophyidae Ohdner, 1914 is a large group of small intestinal trematodes of 22 genera (Yamaguti, 1958). The type genus of this family is *Heterophyes* Cobbold, 1886, which has a characteristic sucker-like genital apparatus called genital sucker. Other morphological characteristics of this family are the body surface beset with scale-like spines and distinctive genital apparatus of various shape, size and location (Witenberg, 1928).

A few kinds of snail hosts are known as the first intermediate hosts and some fresh water or brackish water fishes play the roles of their second intermediate hosts (Komiya, 1965). And the final hosts are the avian and mammalian vertebrates. These worms can be prevalent in the areas where the 3 kinds of hosts are closely related each other ecologically. And they distribute world-widely, including Europe, Africa, Asia and America (Yamaguti, 1958).

In Korea, *Metagonimus yokogawai* is the well-known one of these flukes. Its distribution, life cycle and medical importance have been studied by many workers up to present. However, the distribution of *Pygidiopsis* (Chun, 1963), *Centrocestus* (Choi et al., 1964), *Stellantchasmus* (Seo et al., 1979) and *Heterophyes* (Seo et al., 1980) had ever been recorded other than *M. yokoga-*

*wai*. Recently, Seo et al. (1981) detected the human cases infected with *Pygidiopsis* and *Heterophyes* for the first time in Korea.

To understand more precisely the source of human infection, mode of transmission, geographical distribution, etc. of *Pygidiopsis* and *Heterophyes*, it is necessary to do some survey on the metacercarial infection in the brackish water fishes in Korea. For this purpose, the brackish water fishes from 14 coastal areas were examined for the heterophyid metacercariae.

### MATERIALS AND METHODS

**1. Detection and collection of metacercariae:** As shown in Table 1, 2 and 3, 59 *Mugil cephalus* from 14 areas, 52 *Acanthogobius flavimanus* from 5 areas and 24 *Lateolabrax japonicus* from 5 areas were collected during a period of 6 months from May to October, 1980. They were examined for the infection status by heterophyid metacercariae either by muscle compression method or peptic digestion technique. The procedures of detecting and collecting metacercariae were carried out under a dissecting microscope.

Some of the isolated metacercariae were excysted under cover slip pressure and fixed with 10% formalin. They were stained with Semichon's acetocarmine and observed.

**2. Experimental infection and worm recovery:** The metacercariae of *Pygidiopsis* were infected to 10 albino rats. They were fed to rats through a polyethylene capillary tube which was

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inserted into stomach under ether anesthesia.

Adult worms were obtained from the small intestines of rats after various durations of infection; 18 hours, 1, 2, 3, 4, 5, 7, 10, 20, 28 and 30 days. In warm saline solution, the isolated small intestines were separated into duodenal, jejunal and ileal parts, and the lumen was opened along the mesenteric border. Moving worms were collected from the intestines under a dissecting microscope.

The worms were fixed with 10% formalin under a cover slip. They were stained with Semichon's acetocarmine and observed.

The metacercariae of *Heterophyes* were infected to 5 albino rats and adult worms were harvested from their intestines after 8~16 days by the same method described above.

Stool specimens of the rats were examined daily by formalin-ether concentration technique for the eggs.

## RESULTS AND WORM DESCRIPTION

### 1. The metacercariae of *Pygidiopsis*

**summa:** Metacercariae of *Pygidiopsis* were found in 17 *M. cephalus* and an *A. flavimanus* (Table 1 and 2). No *L. japonicus* was found infected (Table 3). The areas where the fishes were positive for the metacercariae of *Pygidiopsis* were Okku Gun, North Cholla Do; Mokpo City and Yeochun Gun, South Cholla Do and Samchunpo City, South Kyongsang Do (Table 1, 2 and Fig. 1). The positive rates of mullets were from 7% to 100% by area. Metacercarial burden in a fish

Table 1. Detection of metacercariae from *Mugil cephalus*

Area	No. of examined	No. of positive for		No. of metacercariae in a fish (mean)	
		<i>Pygidiopsis</i>	<i>Heterophyes</i>	<i>Pygidiopsis</i>	<i>Heterophyes</i>
Kangwha	3	0	0	0	0
Yongyu	2	0	1	0	87
Seosan	6	0	0	0	0
Boryong	5	0	0	0	0
Kunsan	5	0	0	0	0
Okku	7	7	1	201-4, 954(2, 094)	2
Mokpo	6	6	6	2-112(52)	5-38(23)
Yeochun	5	3	3	17-221(85)	2-67(29)
Namhae	1	0	0	0	0
Samchunpo	14	1	0	2	0
Ulsan	5	0	0	0	0
Pohang	6	0	0	0	0
Yeongdok	3	0	1	0	49
Kangneung	1	0	0	0	0
Total	59	17	12	2-4, 954	2-87

Table 2. Detection of metacercariae from *Acanthogobius flavimanus*

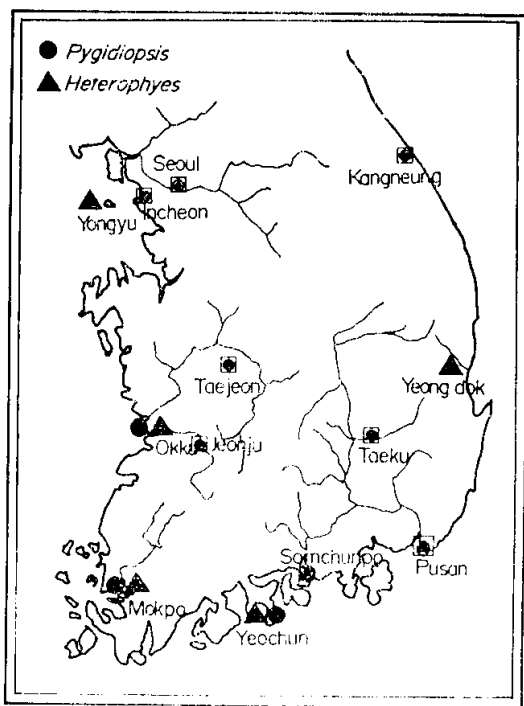
Area	No. of examined	No. of positive for		No. of metacercariae/fish (mean)	
		<i>Pygidiopsis</i>	<i>Heterophyes</i>	<i>Pygidiopsis</i>	<i>Heterophyes</i>
Boryong	2	0	0	0	0
Okku	28	0	0	0	0
Mokpo	12	1	1	9	16
Yeochun	5	0	1	0	1
Namhae	5	0	0	0	0
Total	52	1	2	9	17(9)

**Table 3.** Detection of metacercariae from *Lateolabrax japonicus*

Area	No. of examined	No. of positive for	
		<i>Heterophyes</i>	<i>Pygidiopsis</i>
Kunsan	3	0	0
Okku	7	0	0
Samchunpo	10	0	0
Ulsan	3	0	0
Kangneung	1	0	0
<b>Total</b>	<b>24</b>	<b>0</b>	<b>0</b>

was from 2 to 4,954. The great majority of the metacercariae were isolated from gills of mullets and very few from muscle (Table 4). The metacercariae could not be found from scale, fin and peritoneal cavity. The only *A. flavimanus* that was positive for the metacercariae of *Pygidiopsis* was obtained from Mokpo City (Table 2). The location of metacercariae in the fish was not checked.

**Description of metacercariae of *P.***



**Fig. 1.** Distribution map of *Pygidiopsis* and *Heterophyes* metacercariae.

**summa:** The metacercarial cyst was globular, round to elliptical, measured 0.19~0.29×0.16~0.29mm (Fig. 2). Cyst wall 0.003~0.005mm thick.

Oral and ventral suckers well-developed, excretory vesicle X-shaped and filled with bluish-black excretory corpuscles. Excysted metacercaria (Fig. 3) was slender and tapered anteriorly, and blunt and round posteriorly, 0.252~0.438 mm long and 0.133~0.199mm wide in stained specimens. Beset with small scale-like spines densely on the body surface except for the posterior one third of body. Dark pigments are present in both sides of pharynx. Oral sucker subterminal and measured 0.034×0.028mm,

**Table 4.** Location of *Pygidiopsis* metacercariae in 17 infected mullets

	Location	
	Gill	Muscle
No. of infected mullets	16	3
No. of metacercariae, range	2-4,954	1-16
No. of metacercariae, mean	950	7

prepharynx 0.038mm long, pharynx, well-developed and muscular, 0.024mm long and 0.021 mm wide and esophagus 0.044mm long. Intestinal bifurcation was near anterior one third body line and ceca terminated at the level of testes. Ventral sucker, round and 0.034×0.027 mm in diameter, near midline in the equatorial body portion. Genital apparatus was crescent form, neighbouring left anterolaterally to ventral sucker. Testes, already developed, lying symmetrical in posterior body end, measured 0.041×0.028mm. They pressed the excretory vesicle bilaterally and made it X-shaped. Primordial cell mass of ovary was stained just anteriorly to the excretory vesicle.

**2. The metacercariae of *H. heterophyes nocens*:** As shown in Table 1 and 2, *Heterophyes* metacercariae were isolated from 12 mullets and

2 *A. flavimanus*. The areas where the positive fishes were collected were mapped in Fig. 1. They were Yongyu Do, Ongjin Gun, Kyonggi Do; Okku Gun, North Cholla Do; Mokpo City and Yeochun Gun, South Cholla Do and Yeongdok Gun, North Kyongsang Do. The positive rates of mullets varied from 14% to 100% by area. The metacercarial burden was in range of 2~87 (mean, 31). Most of them were collected from muscle of mullets (Table 5). The number of metacercariae isolated from *A. flavimanus* was 1~16, mean 9, and the location in the fish was not observed. The morphological characteristics and measurements of metacercariae of *Heterophyes* were exactly same with those of Seo et al. (1980), and identified as *H. heterophyes nocens*.

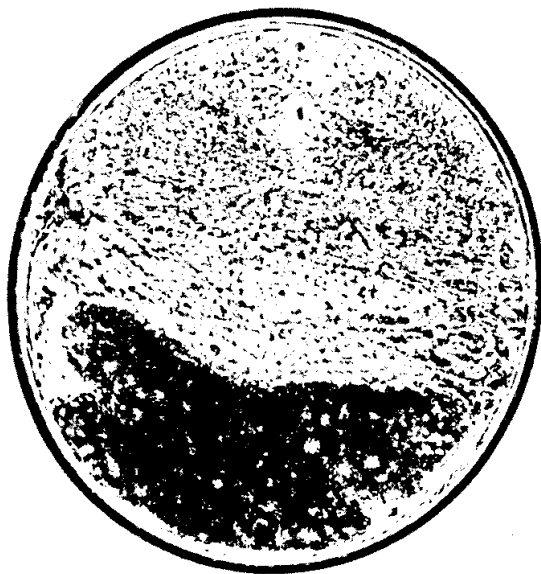
**3. On the experimental infection and development of *Pygidiopsis*:** Recovery rates

**Table 5.** Location of *Heterophyes* metacercariae in 12 infected mullets

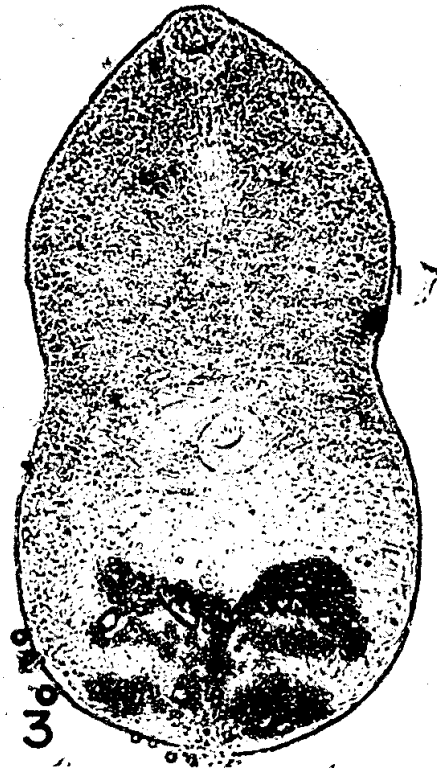
	Location	
	Muscle	Gill
No. of infected mullets	12	3
No. of metacercariae, range	2-87	1-5
No. of metacercariae, mean	30	3

of adult worms 30~53% in the first 20 days and decreased to 1% 30 days after infection. Total 9,030 metacercariae were infected to 10 albino rats and 1,408 (16%) adult worms of various age were harvested from small intestines; 1,353 (96.1%) out of them were collected from jejunum and 42 (3.9%) from ileum (Table 6).

The body size increased in the first three days and no more after then up to 30 days (Table 7). As for the worm development, ovary and vitelline follicles became distinctive within 18 hours.



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3

Fig. 2. Metacercaria of *Pygidiopsis summa*,  $\times 180$ .

Fig. 3. Excysted metacercaria of *Pygidiopsis summa*,  $\times 120$ .

**Table 6.** Results of worm recovery from infected rat intestine

Duration of infection	No. of MC* challenged	No. of recovered worms		
		Jejunum	Ileum	Total(%)
18 hours	300	91	0	91(30)
2 days	300	108	1	109(36)
3 days	300	156	1	157(52)
4 days	300	120	12	132(44)
5 days	1,000	372	1	373(37)
7 days	500	255	7	262(52)
10 days	314	48	3	51(16)
20 days**	500	156	6	162(32)
28 days***	3,000	60	0	60( 2)
30 days****	1,016	0	11	11( 1)
Total	9,030	1,353	42	1,408(16)

\* Metacercariae

\*\* Eggs were found from stool, firstly on the 14th day.

\*\*\* Eggs were found from stool, firstly on the 7th day.

\*\*\*\* Eggs were found from stool, firstly on the 9th day.

Seminal vesicle and receptacle were visible on the second day, and a few eggs appeared firstly in uterus on the second day and filled the uterus 4~5 days after infection.

The eggs were found in stool of 3 rats out of 10. It was 7 days after infection that eggs were found firstly by daily examination. They measured 0.021~0.022×0.011~0.014mm (mean 0.021×0.013mm). They were ovoid in shape and thick shelled, and yellowish brown and contained mature miracidia.

Adult worms were identified as *P. summa*, which were identical with those of Seo et al. (1981) in morphology and measurements.

## DISCUSSION

Present study revealed some foci of *Pygidiopsis* and *Heterophyes* infection along the coastal areas in Korea. The areas are all brackish water zones and the above two flukes tend to be prevalent in same zones. The fishes caught in Okku Gun, North Cholla Do were most heavily infected by the metacercariae, out of the 4 newly recovered areas of *Pygidiopsis* infection. And the authors detected human cases of *Pygidiopsis* and *Heterophyes* infection there (Seo et al. 1981) incidentally.

A kind of brackish water snail, *Tympanotonus* sp., which was known as the first intermediate host of *Heterophyes* and *Pygidiopsis* in Japan (Ochi, 1931), can be found in those areas though not proved as the first intermediate host yet. *M. cephalus* was already reported as the second intermediate host of *Heterophyes* (Seo et al., 1980) and *Pygidiopsis* (Chun, 1963) in Korea. As for the location of metacercariae in the mullets, the great majority of *Pygidiopsis* metacercariae were collected from gill and those of *Heterophyes* from muscle. And this study proved that *A. flavimanus* also played the role of the

**Table 7.** Measurements of worm by various duration of infection in stained specimens

Duration of Infection	No. of measured worms	Body size(mm)	
		Length(mean)	Width(mean)
Metacercaria	10	0.252~0.438(0.326)	0.133~0.199(0.160)
18 hours	10	0.259~0.365(0.319)	0.126~0.199(0.172)
2 days	15	0.318~0.464(0.366)	0.199~0.225(0.217)
3 days	8	0.511~0.663(0.616)	0.239~0.285(0.261)
4 days	13	0.398~0.656(0.566)	0.252~0.365(0.316)
5 days	12	0.438~0.683(0.591)	0.265~0.385(0.332)
7 days	10	0.398~0.564(0.498)	0.239~0.345(0.307)
20 days	10	0.451~0.630(0.540)	0.265~0.371(0.314)
30 days	10	0.398~0.656(0.533)	0.153~0.365(0.300)

second intermediate host of *Pygidiopsis* and *Heterophyes* as well as *M. cephalus* in Korea. However, its metacercarial burden was not so high as that of *M. cephalus*.

Rat experiment showed that growing and maturing of *Pygidiopsis* were very rapid. The eggs were found in uterus on the second infection day firstly. This finding suggested the possibility of in vitro culture of this fluke, though not tried at present. And the rat seemed to be a not so suitable host of *Pygidiopsis* considering the worm recovery rate on the 30 days after infection. The eggs were not so easily found, even from the rats which were infected with hundreds of worms. This finding was also noted in human cases (Seo et al., 1981). Therefore, it can be said that the case harbours a hundred of worm at least when he is egg positive in stool examination.

The infection of these two flukes seems not so medically significant in Korea because the metacercarial burden in the fish host was low in muscle and the treatment is easy. The human cases of pygidiopsiasis in Okku area were proved to be infected eating raw gills of mullets. The metacercariae of *Pygidiopsis* concentrated in gills of mullets. The medical significance of heterophyid fluke infections including pygidiopsiasis lies in the situation that it would be possibly regarded as clonorchiasis by detecting the egg in stool examination. Therefore, it became more important to differentiate the small trematode eggs in stool examination, especially heterophyid eggs from those of *C. sinensis*.

## SUMMARY

The metacercarial infection of brackish water fishes was studied to reveal out the involved species, and their distribution in Korea, from May to October, 1980. Three species of fishes were collected from 14 coastal areas and examined by either muscle compression method or

peptic digestion technique. Heterophyid metacercariae were isolated and identified to be *Pygidiopsis summa* and *Heterophyes heterophyes nocens* through experimental infection to rats. The results are as follows.

1. A total 17 out of 59 examined *Mugil cephalus* and one out of 52 *Acanthogobius flavimanus* were found infected with the metacercariae of *Pygidiopsis*. The metacercariae were isolated mainly from gill of mullets and only a few from muscle. The areas where the infected fishes were collected were Okku Gun, North Cholla Do; Mokpo City and Yeochun Gun, South Cholla Do and Samchunpo City, South Kyongsang Do.

2. Metacercariae of *Heterophyes* were isolated from 12 out of 59 *M. cephalus* and 2 out of 52 *A. flavimanus*. They were found mainly from muscle of mullets, and only a few from gill. The positive loci were Yongyu Do, Ongjin Gun, Kyonggi Do; Okku Gun, North Cholla Do; Mokpo City and Yeochun Gun, South Cholla Do and Yeongdok Gun, North Kyongsang Do.

3. The morphology of *Pygidiopsis* and *Heterophyes* metacercariae was described.

4. Experimental infection of *P. summa* to rats showed rapid sexual maturity of the worm. Eggs began to appear within 2 experimental days, and appear in stool within 7 days.

From this study, it was turned out that the distribution of *Pygidiopsis* and *Heterophyes* was more ample than previously considered, though their clinical significance should be checked further.

＝국 문 초 록＝

한국의 장흡충류에 관한 연구

## IV. 異形吸蟲과 *Pygidiopsis*

피낭유충의 국내분포

서울대학교 의과대학 기생충학교실 및 풍토병연구소

徐丙高 · 洪性台 · 蔡鍾一 · 趙昇烈

승어등 반염수어가 매개하는 腸吸蟲에 대해서는 국

내 일부지역에서 피낭유충을 발견한 보고가 있고, 최근에는 異形吸蟲과 *Pygidiopsis*의 인체감염례도 발견되었다. 이 연구에서는 異形吸蟲科 腸吸蟲중 국내 분포種과 지리적인 분포에 관심을 갖고 1980년 5~10월에 전국 14군데의 해안에서 송어 59마리, 문절망둑 52마리, 농어 24마리를 수집하여 근육압평법과 인공소화법으로 피낭유충의 감염상태를 조사하였다. 그 결과는 다음과 같다.

1. 송어의 문절망둑에서 *Pygidiopsis*와 *Heterophyes*의 피낭유충을 분리할 수 있었다. *Pygidiopsis*의 피낭유충은 전북 옥구, 전남 목포 및 여천, 경남 삼천포등 4개 지역産 17마리에서, 주로 아가미에서 분리되었고, *Heterophyes*의 피낭유충은 경기 용유도, 전북 옥구, 전남 목포 및 여천, 경북 영덕등 5군데의 송어 12마리의 근육에서 주로 발견되었다.

문절망둑 체내의 감염부위는 확인하지 못하였고, 농어에서는 확인 가능한 피낭 유충을 분리할 수 없었다.

2. *Pygidiopsis*의 피낭유충은 球型에 크기가 0.19~0.29×0.16~0.29mm에 배설낭이 X字 모양이었다. 환귀에 감염시킨 층은 대부분이 空腸에서 회수되었고, 성충의 크기는 3일에, 산란은 7일 이내에 완성됨을 관찰하였다. 층의 형태와 계측치에 의해 *P.summa*로 동정하였다.

3. 異形吸蟲은 生殖盤이 뚜렷하였고 rodlets의 수에 의해 *H. heterophyes nocens*로 동정하였다.

이상과 같이 *P.summa*와 *H. heterophyes nocens*의 피낭유충을 국내 몇 군데에서 발견하였고, 이들이 반염수어를 통해서 인체감염을 일으킬 수 있을 것으로 생각되었다.

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