

Growth and Development of *Pygidiopsis summa* in Rats and Mice with a Supplementary Note on Its Morphological Characters

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INTRODUCTION

Pygidiopsis summa (Trematoda; Heterophyidae) is a small intestinal fluke of birds and mammals, including man, in Far Eastern countries such as Korea and Japan (Ito, 1964; Yokogawa *et al.*, 1965; Seo *et al.*, 1981a). This species was first found by Nishio (1915) from the dogs fed on the infected mullets. Later it was described as a new species (Onji *et al.*, 1916) with differential characters from the pre-existing Egyptian type, *P. genata* (Looss, 1907), such as larger body and egg sizes and reverse size ratio of oral and ventral suckers. Further morphological differences have not been described between the two species.

The life history of *P. summa* was successfully studied (Ochi, 1931; Ito 1964); a kind of brackish water snail (*Tympanotonus microptera*) and several kinds of brackish water fishes (*Mugil cephalus*, *Acanthogobius flavimanus*, *etc.*) are known to be its first and second intermediate hosts, and mice, rats, cats and dogs are experimental or natural final hosts. As for the worm development in animal hosts, however, only a brief observation was made by Ochi (1931) and Seo *et al.* (1981b), leaving a necessity of further studies.

Therefore, the present study was performed to observe the growth and development of *P. summa* in experimental rats and mice, and to give a supplementary note on some morphological

characters differed from *P. genata*.

MATERIALS AND METHODS

Metacercariae of *P. summa* (Fig. 8) were obtained from young mullets (*Mugil* sp.), 15~20cm in length, caught from the waterways within a large salt farm located at a western coastal area of Okku-gun, Jeonrabuk-do, where human pygidiopsiasis cases were reported (Seo *et al.*, 1981a). Several hundreds of metacercariae were collected from the head (gills) and esophago-intestine of each mullet, by means of artificial digestion technique.

Each of 21 rats (Wistar) and 21 mice (ICR) of either sex, was fed with 1,000 metacercariae through a gavage needle. They were divided into 7 groups of equal numbers according to the scheduled durations of infection; 3, 5, 7, 10, 14, 21 and 28 days. After each time had passed the rats and mice were sacrificed by cervical dislocation and *P. summa* worms were collected from their small intestine under a dissecting microscope.

The specimens of various ages of infection were fixed in 10% formalin under a slight pressure of a cover slip and observed the details of internal structures such as suckers, intestines and genital organs, and 10 specimens for each group were measured. Several worms of each age were stained with Semichon's acetocarmine so as to identify the shape and location of genital organs.

RESULTS

1. Growth and Development of *P. summa* in Rats and Mice

The chronologic growth and development of *P. summa* in rats and mice were characterized by early rapid growth up to 10 post-infection days followed by stationary growth thereafter (Figs. 1-6 & 7). At metacercarial stage, 10 excysted specimens measured 0.25~0.44mm (average 0.33mm) long and 0.13~0.20mm (0.16 mm) wide. They, after infection to both animals, rapidly grew in their length and width to become 0.40~0.49mm (0.43mm) and 0.24~0.32mm (0.27mm) at 3 days, 0.43~0.63mm (0.53mm)

and 0.29~0.34mm (0.32mm) at 5 days, 0.43~0.55mm (0.50mm) and 0.25~0.32mm (0.28mm) at 7 days and 0.53~0.82mm (0.68mm) and 0.31~0.39mm (0.33mm) at 10 days. Later than that time the mice revealed no more worms in their small intestine, so that only worms recovered from the rats were observed and measured (Fig. 7 & Table 1). Later than 10 days of infection, they appeared to have attained minimum growth, and revealed the length and width of 0.57~0.85mm and 0.27~0.43mm respectively. The growth of each organ was approximately of the same pattern as that of their total body (Table 1).

Male and female genital organs revealed their characteristic growth according to increase of the

Table 1. Measurements of *P. summa* recovered from albino rats and mice

Organs	Range(average) of measurements* of worms(mm)					
	3-day old		10-day old		14-day old	
	Rats	Mice	Rats	Mice	Rats	Mice**
Body length	0.40~0.49 (0.43)	0.36~0.53 (0.45)	0.53~0.82 (0.68)	0.54~0.62 (0.58)	0.58~0.85 (0.71)	—
Body width	0.24~0.32 (0.27)	0.23~0.31 (0.26)	0.29~0.39 (0.33)	0.33~0.39 (0.35)	0.29~0.41 (0.35)	—
Oral sucker	0.043~0.054 (0.049)	0.046~0.059 (0.051)	0.054~0.072 (0.061)	0.054~0.067 (0.059)	0.051~0.067 (0.057)	—
Prepharynx	0.013~0.038 (0.023)	0.008~0.027 (0.021)	0.013~0.094 (0.039)	0.016~0.027 (0.021)	0.029~0.107 (0.050)	—
Pharynx	0.038~0.067 (0.047)	0.035~0.062 (0.050)	0.038~0.054 (0.047)	0.048~0.054 (0.052)	0.038~0.048 (0.043)	—
	×0.019~0.027 (0.023)	0.016~0.030 (0.023)	0.021~0.040 (0.034)	0.024~0.040 (0.033)	0.021~0.040 (0.035)	—
Esophagus	0.016~0.062 (0.037)	0.021~0.043 (0.033)	0.038~0.134 (0.069)	0.035~0.059 (0.046)	0.021~0.094 (0.068)	—
Ventral sucker	0.054~0.067 (0.061)	0.048~0.062 (0.058)	0.062~0.075 (0.069)	0.064~0.075 (0.071)	0.064~0.075 (0.070)	—
	×0.040~0.054 (0.045)	0.032~0.046 (0.042)	0.051~0.070 (0.061)	0.054~0.062 (0.055)	0.051~0.067 (0.058)	—
Genital apparatus	0.048~0.080 (0.066)	0.054~0.067 (0.059)	0.054~0.078 (0.060)	0.080~0.102 (0.084)	0.059~0.067 (0.063)	—
	×0.021~0.040 (0.029)	0.019~0.032 (0.027)	0.024~0.040 (0.031)	0.027~0.054 (0.041)	0.027~0.040 (0.031)	—
Ovary	0.032~0.067 (0.049)	0.035~0.067 (0.050)	0.048~0.094 (0.059)	0.054~0.094 (0.070)	0.067~0.080 (0.072)	—
Testes	0.075~0.107 (0.090)	0.059~0.102 (0.088)	0.080~0.134 (0.104)	0.102~0.147 (0.115)	0.094~0.134 (0.103)	—
	×0.048~0.067 (0.054)	0.054~0.067 (0.063)	0.051~0.080 (0.061)	0.054~0.094 (0.071)	0.056~0.080 (0.062)	—
Uterine eggs	0.019~0.024(0.022) × 0.011~0.013(0.012)					

* 10 unstained, fresh worms immediately after fixation were measured for each group.

** No worms were recovered from the mice.

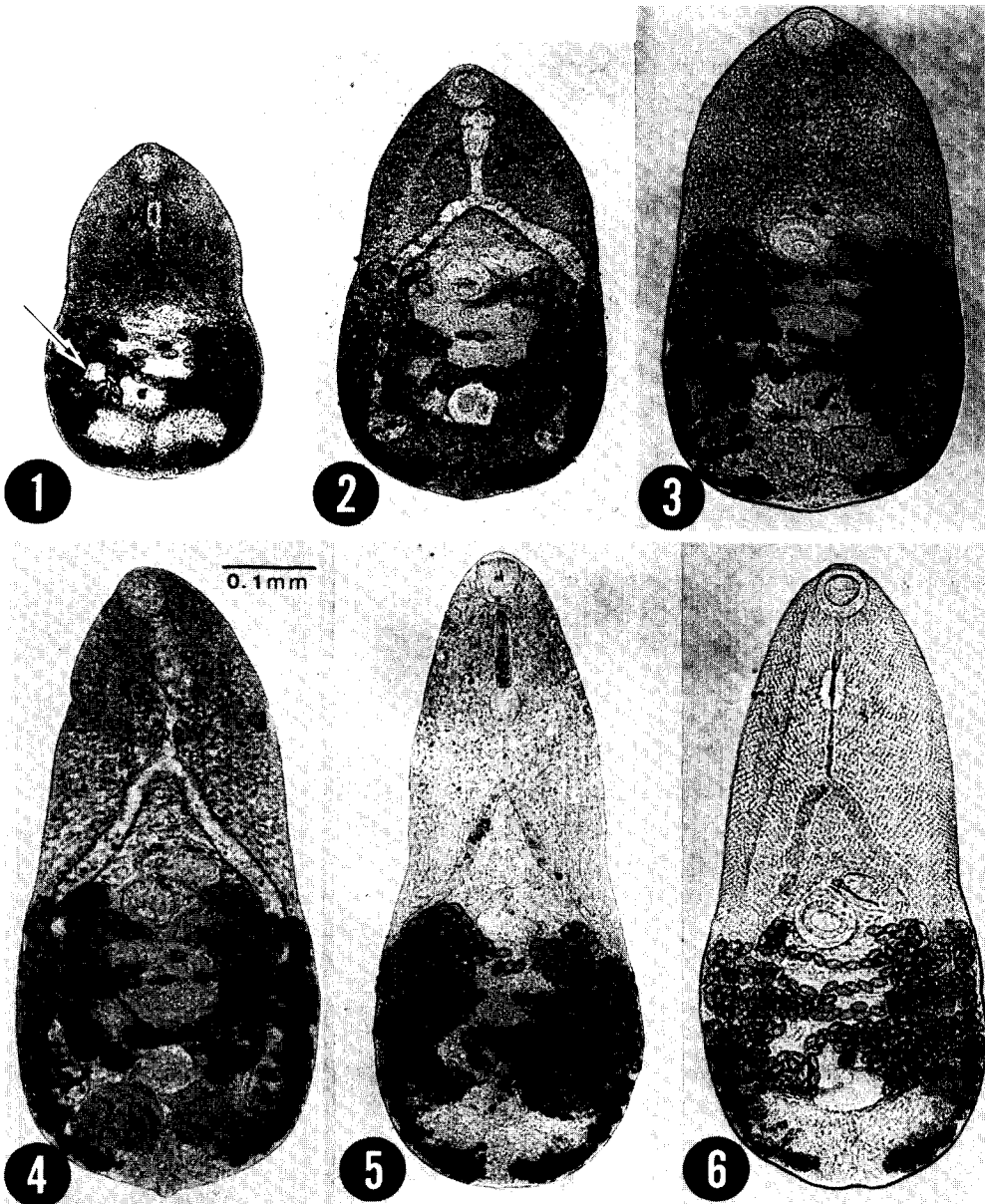


Fig. 1-6. Chronologic development of *P. summa* in rats and mice(unstained specimens after fixation).

1. A 3 day old worm. All of genital organs are already formed and eggs are seen, but vitellaria are not fully differentiated into groups of follicles. 2. A 5 day worm. Eggs are increased in number and vitellaria begin to form groups. 3. A 7 day old worm. Eggs are greatly increased in number and vitellaria appear to have been fully differentiated. 4. A 10 day worm. Body shows its full-grown size and all organs are in their maturity. 5. A 14 day worm. Many eggs are retained. 6. A 21 day worm. Note a little decreased number of eggs.

age of infection. The ovary was already seen in its metacercarial stage, though only as a primordial cell mass, which became morphologically distinct at the age of 3 or 5 days, locating in

the middle, submedian field of posterior half body(Figs. 1 & 2). Thereafter it grew in size up to 28 days. The seminal receptacle was not visible in metacercariae but at 3 days of

infection it appeared just in front of two testes and already contained many sperms. Vitellaria were also developed at 3 days but most specimens revealed not fully differentiated forms (Fig. 1). They were distinctly divided into 6~9 groups of follicles on each side after 5 days (Fig. 2~6). Uterine tubules with eggs were seen in many worms aged 3 days, but a few were not containing eggs. The number of uterine eggs remarkably increased up to 7 days of infection, however, it remained constant after 10~14 days and decreased a little after 21 days.

Testes were recognizable, as a primordial stage, in metacercariae, and they revealed their mature morphology after 3 days of infection in animals. Two lenticular-shape gonotyls, right and left ones, armed each with 5~6 and 7~9 longitudinally arranged, minute spines, were seen in 3 day specimens (Fig. 10), and its morphology became distinct after 5 days (Fig. 11).

2. Brief Description of Morphological Characters of Adult *P. summa*

This description was chiefly based on twenty fully mature worms recovered from rats and mice after 10 days of infection (Figs. 4 & 9), but other 150 specimens of various infection ages were also referred.

Body tapering anteriorly and bluntly ending posteriorly. When living, lateral margins of their body curved ventralwards to reveal a characteristic scaphoid shape with a ventral cavity. Oral sucker subterminal and beset with a single crown of circumoral spines, only in life. Prepharynx present but length variable according to extent of body contraction. Pharynx well developed and remnants of two eye spots distinctly seen on bilateral sides of pharynx. Esophagus longer than prepharynx. Two ceca extending down to anterior margin of testes and each ended bluntly with a swollen end. Ventral sucker transversely elliptical but always a little obliquely and sub-medially located near the middle portion of body. Genital apparatus located at the left anterolateral portion of ventral sucker and a little elongated or crescentic in shape, internally bearing two

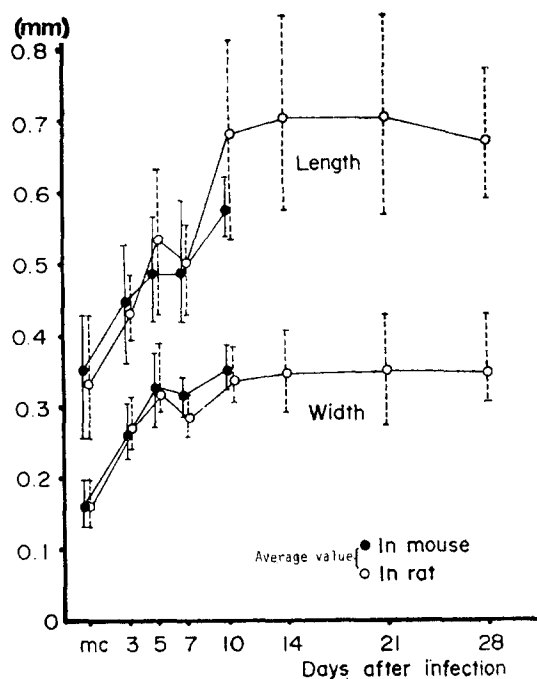


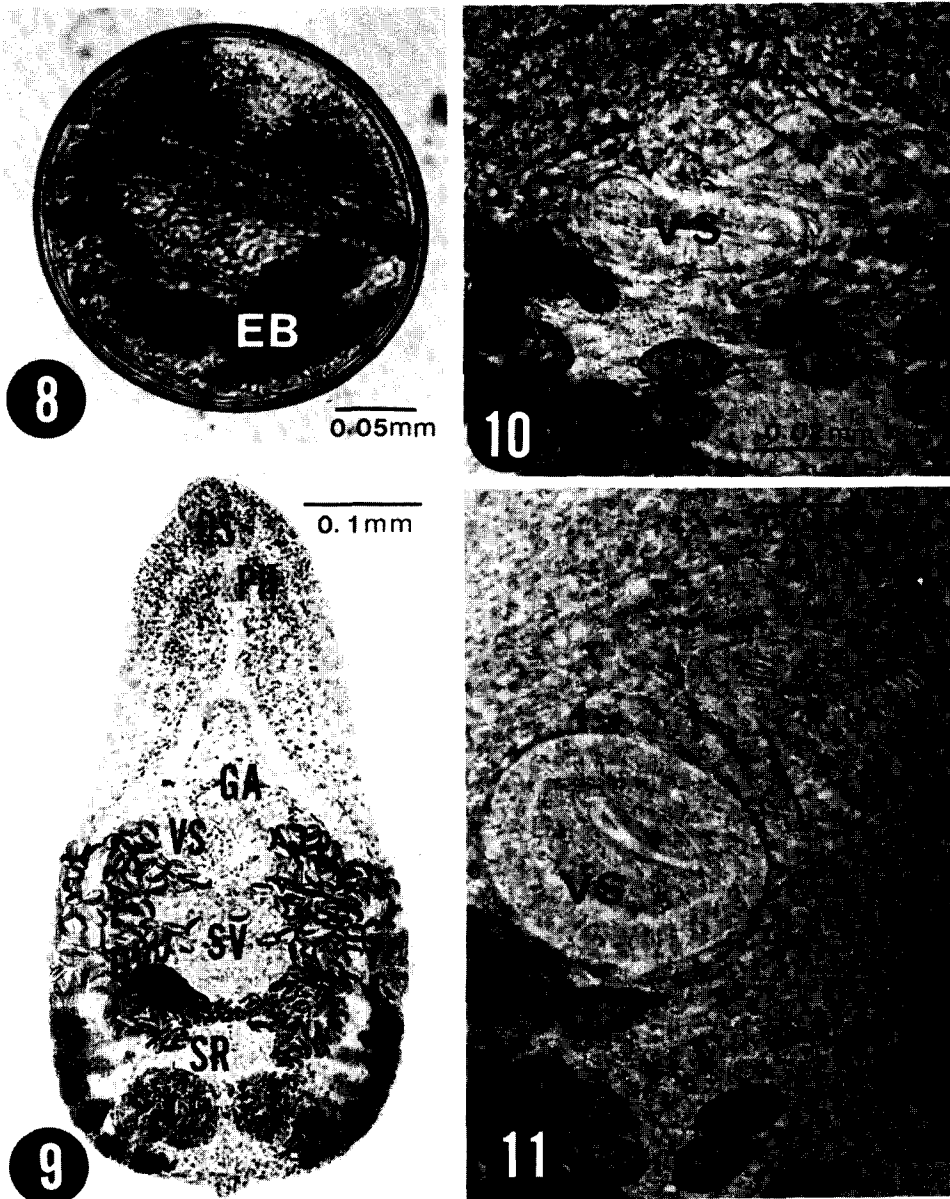
Fig. 7. Growth curves of *P. summa* in rats and mice (Worms older than 10 days werenot recovered from the mice).

groups of small spines (right and left ones; Fig. 11). Number of spines 5~6 on the right side and 7~9 on the left. Seminal vesicle bilobed with a constriction, frequently C-form, and containing sperms. Vitelline glands formed 6~9 groups of follicles. Intrauterine eggs small (0.019 ~0.024mm long), ovoid to pyriform, relatively thick-shelled with clear and smooth outer surfaces and sometimes bearing shoulder rims.

DISCUSSION

The growth and development of *P. summa* in laboratory animals have seldom been subjected to a detailed study. Ochi (1931) obtained 4 hour to 5 day juvenile worms from 4 mice, 20 day mature ones from a kitten, and 10 day, 30 day and 60 day old worms from 3 puppies, but he did not describe their morphological development.

Seo *et al.* (1981b) recovered total 1,408 specimens of various ages of infection (from 18 hours to 30 days) from 10 rats and briefly described on the development of worms. According to



- Fig. 8.** A metacercaria of *P. summa* collected from a mullet. The X-shaped excretory bladder(EB) is a characteristic morphology.
- Fig. 9.** A fully mature *P. summa* recovered 10 days after infection to a rat host. Acetocarmine stained specimen(OS; oral sucker, PH; pharynx, VS; ventral sucker, GA; genital apparatus, SV; seminal vesicle, and T; testes).
- Fig. 10.** Magnification of the middle portion of a 3 day old worm. Ventral sucker(VS) and genital apparatus or gonotyl armed with 7 minute spines on the left side (arrow heads) and several intrauterine eggs are seen.
- Fig. 11.** *Ibid*, a 7 day worm. The gonotyls formed two groups and each armed with 5(right side) and 9(left) spines (arrow heads).

them, the ovary and vitelline follicles were distinctly seen at 18 hours of infection, and the seminal vesicle, seminal receptacle and a few eggs were seen at 2 days. In the rat stool, the eggs were found after 7 days of infection. All of their observations were compatible with the results of the present study. By this study, it was confirmed that the sexual maturity of *P. summa* was as rapid as to complete within 3~5 days in rats and mice, though its full growth in size was attained later than 10 days.

Such a rapid growth and maturation of *P. summa* in final hosts seems one of common characteristics of heterophyid flukes since similar results have been reported in *Metagonimus yokogawai* (Ito, 1964; Hong *et* Seo, 1969), *Heterophyes heterophyes nocens* (Seo *et al.*, 1980), and others. But *P. summa* attained its maturity more rapidly than other heterophyids. One of the responsible factors may be the maturity of genital organs in metacercarial stage in the fish host. The excretory bladder of *P. summa* metacercariae is approximately X-form (Fig. 8) which is due to the existence of two testes nearby the right and left sides and of an ovary on the anterior portion. These sexual organs are less distinctly seen in the metacercariae of *M. yokogawai* or *H. heterophyes nocens* (Hong *et* Seo, 1969; Seo *et al.*, 1980). A speculation with this could be that *in vitro* cultivation of *P. summa* metacercariae might be highly successful, so as to be used as a model for the research of *in vitro* cultivation of trematodes.

It seems worthwhile to reconsider some taxonomic problems related to *P. summa* (Onji *et* Nishio, 1916) and the type species, *P. genata* (Looss, 1907). The problems are such as that a few workers have dealt *P. summa* as a synonym of *P. genata* (Kuntz *et* Chandler, 1956), and in Canton, China, a geographically adjacent area to Korea or Japan, *P. genata* was reported from the dog (Faust *et* Nishigori, 1926). Skrjabin (1964) erroneously described one of differential points between the two species was the presence of a small posterior appendage at the oral sucker of *P. summa* while not in case of

Table 2. Some differential characters between *P. summa* and *P. genata*

Item	<i>P. summa</i>	<i>P. genata</i>
Describer	Present authors	Looss(1907) Witenberg(1929)
Length of body*	0.5~0.8mm	0.4~0.7mm
Width of body	0.3~0.4mm	0.2~0.4mm
Oral sucker	0.05~0.07mm	0.03~0.05mm
Ventral sucker	0.06~0.08mm	0.04~0.06mm
size	by 0.05~0.07mm	
shape	transversely elliptical	globular
location	slightly submedian and a little oblique	median
Genital apparatus		
gonotyl	two groups	one group
spines (No.)	5~6(right) 7~9(left)	?
Ceca termination	to anterior border of testes	to level of ovary
	not turning medialwards with bluntly swollen ends	turning dorsomedially ?
Vitellaria	forming 7~9 groups of follicles	forming 5~8 groups of follicles

*Adult specimens

P. genata. Based on several morphological characters of the former, anyway, is of the authors' opinion that *P. summa* is a distinct and valid species.

Several differential points were as listed in Table 2. Firstly, in *P. genata*, there was no description on the presence, and/or number, of spines on gonotyl within the genital apparatus (ventro-genital sac) (Looss, 1907; Ransom, 1920; Witenberg, 1929). However, in the present specimens of *P. summa*, two groups of small spines, 5~6 on the right side and 7~9 on the left, were evidently recognizable (Fig. 11). Secondly, in *P. genata*, the ceca reached only at the level of ovary and they turned somewhat dorsomedially at their terminal portion. In comparison, the ceca of *P. summa* extended down to the anterior of testes and had much distended ends instead of turning medialwards. Thirdly, the ventral sucker of *P. genata* was approximately round and globular in shape while that

of *P. summa* was constantly elliptical in shape, and transversely oblique and a little submedian in its position. The shape and arrangement of vitellaria were also a little different between the two species but it may not be a significant feature.

For the above reasons, infection of the dog in Canton, China (Faust *et* Nishigori, 1926) of *P. genata* seems to have been of *P. summa*. It seems possible that they were not aware of the existence of *P. summa* in Japan (Onji *et* Nishio, 1916 & 1924). The small appendage of oral sucker described by Skrjabin (1964) was not observed in the present specimens, and it seems not a differential point of *P. summa* from *P. genata*. More than 5 other species of *Pygidiopsis* have been described in various countries, as listed by Seo *et al.* (1981a), but comparisons with *P. summa* were not performed.

SUMMARY

The growth and development of *Pygidiopsis summa* were studied in experimental rats and mice, and a special reference was given to its morphological characters differed from the type species, *P. genata*. The metacercariae were obtained from young mullets (*Mugil* sp.), and total 21 rats and mice infected each with 1,000 metacercariae. Worms of various ages of infection, from 3 to 28 days, were subjected to a microscopic observation.

The worms grew rapidly and remarkably in size up to 10 days of infection, to become 0.53~0.82mm long and 0.31~0.39mm wide, but nearly stopped the growth thereafter. Their genital organs developed more rapidly and fully matured within 3~5 days. At 3 days several eggs were found in uterus. The presence of two groups of small spines, 5~6 on the right and 7~9 on the left side of the genital apparatus, was a new finding in this study and considered a distinct character of *P. summa*. The morphology of ventral sucker and intestinal ceca was also different from *P. genata*. This study confirms the validity of the species, *P. summa*.

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==국문초록==

***Pygidiopsis summa*의 흰쥐와 마우스내에서의 성장발육 및
蟲體 형태학적 특징에 대한 검토**

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실험감염된 흰쥐와 마우스에 있어서 *Pygidiopsis summa*의 성장발육 과정을 관찰하는 한편 이 吸蟲의 형태학적 특징으로서 이집트産인 *P. genata*와의 차이점에 대하여 재검토하였다. 被囊幼蟲은 전북 옥구産 어린 송어(*Mugil* sp.)에서 획득하였고 흰쥐 21마리 및 마우스 21마리에 각각 1,000개씩 경구감염시켰고 감염 3일부터 28일까지 蟲體를 回收하여 형태학적 관찰을 시행하였다.

蟲體의 성장발육은 그 크기에 있어서 감염 10일까지의 급속한 성장과 그 후의 성장정지로 특징지을 수 있었고 감염 10일된 성충의 크기는 길이 0.53~0.82mm, 폭 0.31~0.39mm이었다. 한편 蟲體의 生殖器官인 辜丸, 卵巢, 生殖裝置(genital apparatus), 卵黃腺 등은 매우 급속한 성장발육을 보여 감염 3~5일에 모두 성숙하였고 이때 이미 子宮內에는 蟲卵이 形成되어 있었다.

生殖裝置內의 左右에는 각각 5~6(右側)개 및 7~9개의 小棘(small spines)이 이 吸蟲의 새로운 형태학적 특징으로서 관찰되었고, 타원형이며 약간 비스듬히 體 中央線에서 側面으로 치우친 腹吸盤(ventral sucker), 끝이 體 中央部로 꼬부라지지 않고 辜丸 前方까지 뻗어 있으며 팽대되어 끝나있는 蟲體 腸管(ceca), 등이 *P. genata*와는 다른 특징적 형태로 판단되었다. 따라서 *P. summa*는 분명히 *P. genata*와는 다른 別個의 種으로 생각되었다.