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Three morphological types of the genus *Metagonimus* encysted in the dace, *Tribolodon taczanowskii*, caught from the Sumjin River

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Abstract: Three morphological types of the genus *Metagonimus* were found encysted in the dace, *Tribolodon taczanowskii*, caught from the Sumjin River, Korea. They include *Metagonimus* Yokogawa type (*M. yokogawai*), Miyata type, and Koga type according to Saito's classification.

The metacercariae were experimentally fed to rats and hamsters and a total of 1,624 *Metagonimus* worms were recovered from their small intestine. The number of each type was 820 for Yokogawa type, 688 for Miyata type, 46 for Koga type, and 70 undetermined. The major differential keys between Yokogawa and Miyata types were in the position of two testes, distribution of uterine tubules, and size of the eggs, and the keys between Miyata and Koga types were in the position of two testes, distribution of vitelline follicles, and size of the eggs. The validity of Miyata type as a specific level should be retained until more convincing evidences are obtained. Koga type is regarded as a synonym of *M. takahashii*.

The present results reveal that there are 3 morphological types of *Metagonimus* whose larvae are encysted in the dace, *T. taczanowskii*, from the Sumjin River.

Key words: *Metagonimus yokogawai*, morphological types, rats, hamsters, dace (*Tribolodon taczanowskii*), Sumjin Rver.

INTRODUCTION

Metagonimiasis is now regarded as one of the three major trematodiases in Korea along with clonorchiasis and paragonimiasis. Endemic foci of human infections with this fluke have been reported in various parts of Kyongsangnam-do (Yeo and Seo, 1971; Joo and Park, 1982), Jeonranam-do (Soh *et al.*, 1976; Chai *et al.*, 1977; Soh and Ahn, 1978), Cheju-do (Kang *et al.*, 1964), Kangwon-do (Ahn and Ryang, 1988), and eastern coastal areas (Seo *et al.*, 1981).

Taxonomically the genus *Metagonimus* is known to consist of five species, namely *Metagonimus yokogawai* Katsurada (1912), *Metagonimus takahashii* Suzuki, 1930, *Metagonimus minutus* Katsuda, 1932, *Metagonimus katsuradai* Izumi, 1935, and *Metagonimus otsurui* Saito and Shimizu, 1968. The major differential points among the species were 1) habitat in the host,

2) kinds of intermediate hosts, 3) vitelline follicle distribution, 4) extension pattern of uterine tubules, 5) location and size of ovary and seminal vesicle, 6) relative size of oral and ventral suckers, and 7) egg size. However, there have been a number of debates and confusions on the taxonomy of the genus *Metagonimus* (Asada, 1934; Ito, 1964; Saito, 1984). Especially the debates on the validity of *M. takahashii* as a distinct species or not (Takabayashi, 1953; Komiya *et al.*, 1958; Saito, 1972 & 1973) seem not yet completely settled. Furthermore, there was raised a new proposal of *Metagonimus* Miyata type and Koga type, the former of which might be a different species from *M. yokogawai* or *M. takahashii* (Saito, 1984).

In Korea, the great majority of papers on the genus *Metagonimus* have been concerned with *M. yokogawai* (Kang *et al.*, 1964; Seo *et al.*, 1971; Song *et al.*, 1985; Chai and Lee, 1990) and only a few were with other species or types. Chun (1960) reported *M. takahashii* from experimental rabbits fed the metacercariae encysted in *Carassius carassius* from the Nakdong river. Recently Kim *et al.* (1987) reported *Metagonimus* Miyata type (Saito, 1984) encysted in *Opsariichthys bidens*, *Zacco platypus* or other fresh water fish caught from the Geum river.

The present authors have found numerous *Metagonimus* metacercariae from the dace, *T. taczanowskii*, collected from the Sumjin river. After experimental infection of the metacercariae to rats and hamsters, adult worms were recovered from their small intestine. By careful observations of their morphology the flukes could be classified into three different types belonging to the genus *Metagonimus*.

MATERIALS AND METHODS

1. Collection of the metacercariae and infection to rats and hamsters

Metacercariae of the genus *Metagonimus* were isolated from the body portions, *i.e.*, scale, muscle, fin, head and gill, and viscera, of the dace, *Tribolodon taczanowskii*, which were caught from the Sumjin river, Hadong-gun, Kyongsangnam-do. Peptic digestion technique was applied for isolation of the metacercariae from the fish and the isolated metacercariae were counted under stereomicroscopy to prepare the dose to be challenged. A total of 8 experimental animals, 4 rats and 4 hamsters, were infected orally each with 1,000 metacercariae through a gavage needle.

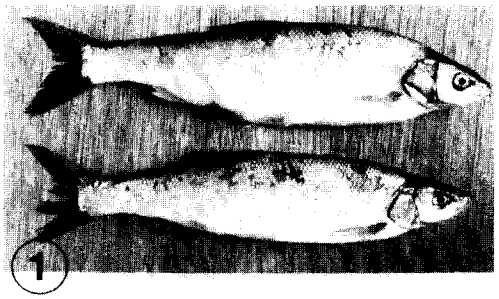
2. Worm recovery from the animals

The rats and hamsters fed the metacercariae were sacrificed by cervical dislocation individually 7~14 days after the infection. The peritoneum was opened and the whole small intestine was resected from the gastric pylorus to the ileocecal portion. The segments of the duodenum, jejunum and ileum were opened longitudinally in 0.85% saline. The worms were harvested from the sediments of the intestinal content, fixed with 10% neutral formalin under cover glass pressure, and stained with Semichon's acetocarmine.

RESULTS

The total number of metacercariae collected from 15 fish, *T. taczanowskii* (Fig. 1), was 8,767. Therefore, the average metacercarial density per fish was 584 (Table 1). The metacercariae were found from various parts of the fish such as scale (Fig. 2), muscle, fin, head and gill, and viscera. However, the majority (8,062; 92.0%) were found from the scale (Table 1). It was hardly possible to discriminate 3 kinds of metacercariae when they were encysted in fish or even after isolation. For this reason, the metacercariae from various parts of the fish were pooled and used for experimental infection to animals.

The recovery rate of *Metagonimus* worms 7~14 days after experimental infection was as shown in Table 2. A total of 424 and 1,200 adult flukes were recovered from the rats and hamsters, respectively. The average worm recovery rates were 10.6% in the rats and 30.0% in the hamsters. The flukes were fully grown



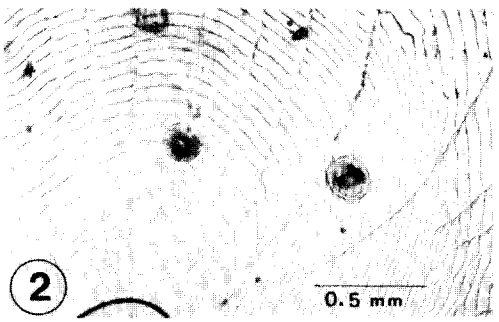


Fig. 1. The dace, *Tribolodon taczanowskii*, caught from the Sumjin river, the second intermediate host of 3 morphological types of *Metagonimus*.

Fig. 2. Two metacercariae of *Metagonimus* encysted on the scale of *T. taczanowskii*.

and matured, containing many eggs in the uterus. Careful observations on the worms after fixation or after staining led us to consider that they are three morphologically different types, which were proposed by Saito (1984) in Japan. They were 820 *Metagonimus* Yokogawa type (*M. yokogawai* in strict sense), 688 *Metagonimus* Miyata type, 46 *Metagonimus* Koga type, and 70 undetermined. The morphological characters of the three types were briefly described.

***Metagonimus yokogawai* Katsurada (1912) (Figs. 3 & 6)**

Table 1. Distribution of *Metagonimus* metacercariae by body portions of *T. taczanowskii*

Body portion	No. metacercariae* collected (%)	Mean No./fish
Scale	8,062(92.0)	537
Muscle	409 (4.7)	27
Fin	212 (2.4)	14
Head & Gill	83 (1.0)	6
Viscera	1 (0.01)	0
Total	8,767	584

*From 15 fish examined

Body oval, flattened, tapering anteriorly, and 682.5～766.5 μm in length and 367.5～378.0 μm in width. Oral sucker subterminal. Ventral sucker larger than oral sucker, laterally deviated near anterior one third level of body. Prepharynx very short. Esophagus moderately long and bifurcating in front of ventral sucker. Two intestinal ceca reaching almost to the posterior end of body. Two testes obliquely adjacent near the posterior end of body. Uterine tubules extending down to middle level of the right (posterior) testis but not overlapping right or left testis nor passing between the two testes. Vitelline follicles distributed in middle to lateral fields of posterior body, 6～8 groups on each side, rarely extending posterior testicular margin (Figs. 3 & 6). Uterine eggs many, elliptical, yellow in color, 25.6～29.4(av. 27.5) μm long and 14.1～17.9 (av. 15.5) μm wide.

***Metagonimus* Miyata type Saito, 1984 (Figs. 4 & 7)**

Body oval, flattened, tapering anteriorly, and measuring 661.7～787.5 μm in length and 378.0～409.5 μm in width. Ventral sucker large than oral sucker, deviated to right side of body.

Table 2. Results of worm recovery* (*Metagonimus*) from experimental rats and hamsters

Exp. animal	No. animals	No. Mc given	No. of worms recovered from			
			duodenum	jejunum	ileum	total(%)
Rat	4	4,000	24	311	89	424(10.6)
Hamster	4	4,000	282	563	355	1,200(30.0)
Total	8	8,000	306	874	444	1,624(20.3)

*At 7～14 days after experimental infection

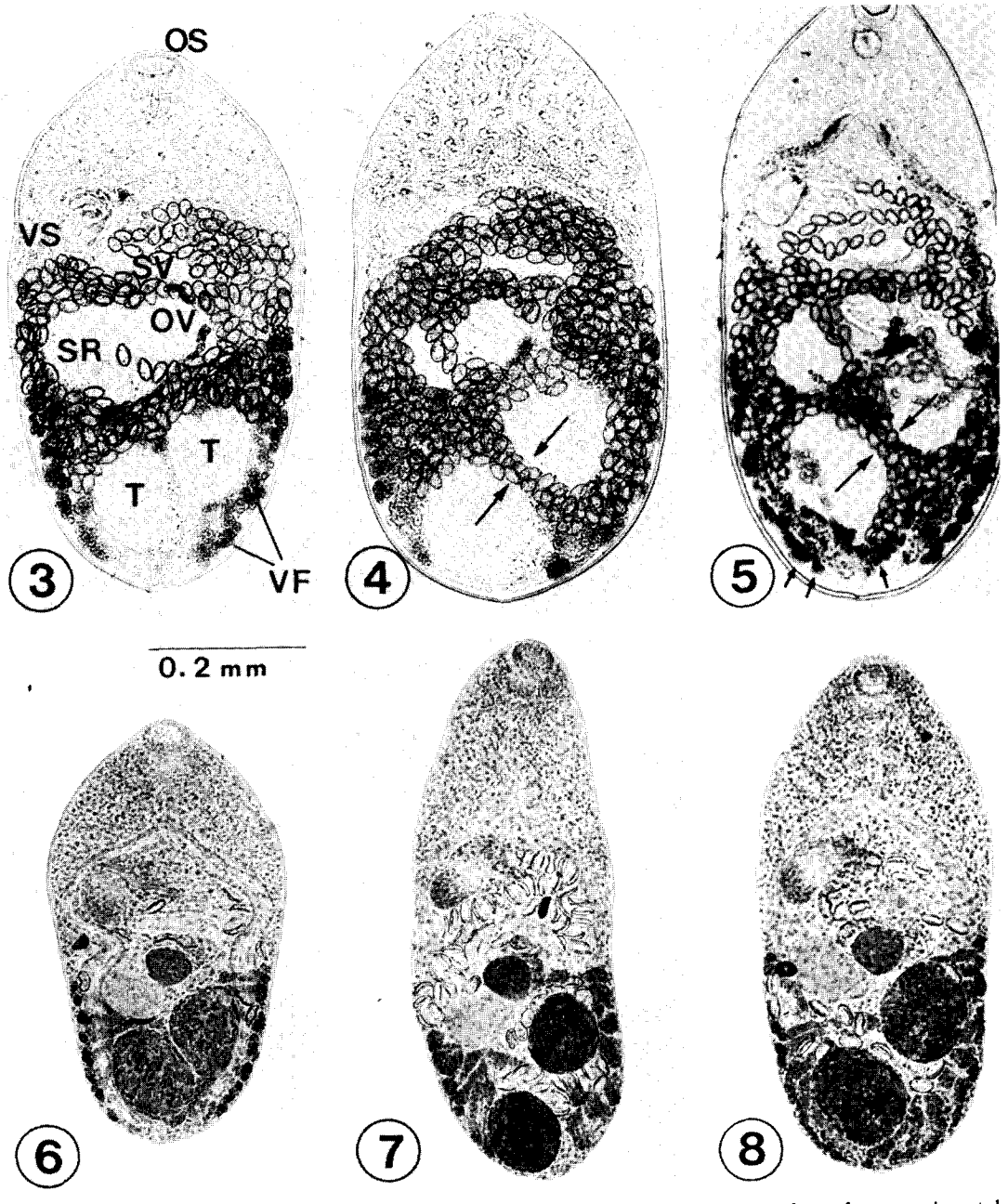


Fig. 3. An adult specimen of *Metagonimus yokogawai* recovered from a rat, 14 days after experimental infection. Note the two adjacent testes(T), and distribution of uterine tubules and vitelline follicles(VF). Oral sucker(OS), ventral sucker(VS), ovary(OV), seminal vesicle(SV) and seminal receptacle(SR). Fresh preparation.

Fig. 4. An adult specimen of *Metagonimus* Miyata type recovered from a rat, 14 days after experimental infection. Note the two separated testes (arrows) and uterine tubule passing through the intertesticular junction. Also note the limited distribution of vitelline follicles near the posteriormost portion of the body. Fresh preparation.

Prepharynx very short. Esophagus moderately long and bifurcating laterally to form two intestinal ceca. Two ceca terminating before the middle level of right testis. Two testes lying obliquely with right one nearly reaching to posteriormost end of body and left one a little separated from the right one. Uterine tubules occupying almost whole posterior field of body, overlapping left (anterior) testis and going across the intertesticular junction. Vitelline follicles distributed in middle to lateral fields of posterior body, 7～8 groups on each side, but not extending beyond the middle portion of right testis, never reaching to posterior end of body (Figs. 4 & 7). Uterine eggs many, elliptical, yellowish brown in color, a little larger than those of *M. yokogawai*, 29.4～33.3 (av. 30.6) μm long and 14.1～19.2 (av. 17.1) μm wide.

***Metagonimus* Koga type, Saito (1984) (Figs. 5 & 8) (=*Metagonimus takahashii* Suzuki, 1930)**

Body a little elongated oval, flattened and leaf-like, 682.5～766.5 μm in length and 367.5～378 μm in width. Ventral sucker larger than oral sucker, deviated to right side of anterior one third body. Prepharynx short. Esophagus moderately long and bifurcating laterally to form two intestinal ceca. Two testes lying obliquely in posterior half of body, separated a little from one another (Figs. 5 & 8). Right testis not reaching to posteriormost portion of body. Uterine tubules occupying nearly all available space of posterior body passing through the intertesticular junction, and frequently overlapping left testis. Vitelline follicles very well developed in middle to lateral fields of posterior body, 8～9 groups on each side, passing through the posterior end of body (Figs. 5 & 8). Uterine eggs many, elliptical, dark yellowish brown in color, 29.7～37.1 (av. 33.5) μm long and 17.9～20.5 (av. 19.1) μm wide.

DISCUSSION

Among the five species of the genus *Metagonimus* reported so far, *M. yokogawai* (the type species), *M. takahashii* and *M. minutus* are differed from *M. katsuradai* and *M. otsurui* in that they have larger ventral sucker compared to their oral sucker. *M. minutus* is different from *M. yokogawai* and *M. takahashii* by its smaller body and egg (23×13 μm) size, larger seminal vesicle, and different kinds of fish host (*Mugil cephalus*) (Katsuta, 1932).

Taxonomic debates on *Metagonimus* have been made in most cases on the synonymy of *M. takahashii* with *M. yokogawai* (Ito, 1964). After *M. yokogawai* was described as the type species from experimental dogs and cats fed the sweetfish, *P. altivelis* (Katsurada, 1912), *M. takahashii* was raised as a new species by that it has larger eggs than *M. yokogawai* and takes cyprinoid fish other than *P. altivelis* as the intermediate host (Takahashi, 1929; Suzuki, 1930). However, the validity of *M. takahashii* has long been put to questions, since there was

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Fig. 5. An adult specimen of *Metagonimus* Koga type (=*M. takahashii*) recovered from a rat, 14 days after experimental infection. Note the two separated testes (large arrows) and uterine tubule passing through the intertesticular junction. A peculiar feature of this type was extensive distribution of vitelline follicles even through the post-testicular (right) portion (small arrows). Fresh preparation.

Fig. 6. A young adult specimen of *M. yokogawai* recovered from a hamster, 7 days after experimental infection. Note the arrangement of two testes and distribution of vitellaria. Acetocarmine-stained.

Fig. 7. An adult specimen of *Metagonimus* Miyata type recovered from a hamster, 8 days after experimental infection. Note the separated testes, and distribution of uterine tubules and vitellaria. Acetocarmine-stained.

Fig. 8. An adult specimen of *Metagonimus* Koga type (=*M. takahashii*) recovered from a hamster, 8 days after experimental infection. Note the arrangement of two testes, and distribution of uterine tubules and vitellaria. Acetocarmine-stained.

no definite morphological difference from *M. yokogawai* except in the size of eggs (Asada, 1934; Takabayashi, 1953; Ochi, 1957; Komiya *et al.*, 1958; Ishii *et al.*, 1959). Therefore, once it was compromised to be called as a variant, *M. yokogawai* var. *takahashii*, by Morishita in 1951 (Ito, 1964), but the problem was not settled after then. In view of *M. takahashii* as a distinct species, Saito (1972) compared morphological characteristics of cercariae, metacercariae and adults of *M. yokogawai* and *M. takahashii* in detail and concluded that they are distinctively different species. Saito (1973) further performed an experimental study on the susceptibility of fish hosts to the cercariae of two species and observed that *P. altivelis* was highly susceptible to *M. yokogawai* whereas *Carrasius auratus* was preferably susceptible to *M. takahashii*. Even after then, the debates on *M. takahashii* seem not completely solved (Miyamoto and Kutsumi, 1978).

Even though we recognize *M. takahashii* as a distinct species, there still remained some problems on the taxonomy of *Metagonimus* found from various kinds of fish hosts or human hosts in Japan and Korea. One of those was on the two kinds of *Metagonimus* metacercariae found from *P. altivelis* in Japan(Miyata, 1944; Saito and Yamashita, 1982). One was *M. yokogawai* but the other was different from *M. yokogawai* nor compatible to *M. takahashii*. Another example was on the *Metagonimus* adults recovered from humans in the riverside areas of the Geum river, Korea, where *P. altivelis* is not available (Kim, 1980). Some of the specimens he recovered were neither *M. yokogawai* nor *M. takahashii* in the morphology of worms and size of the eggs, and he reserved naming of them. Besides this type of *Metagonimus*, there was another group of worms, that also made some taxonomic confusions, which encyst only on the scale of the dace, *Tribolodon* sp., and produce large type eggs (Koga, 1938; Kogame, 1939; Yoshimura *et al.*, 1972).

In this context, Saito (1984) thoroughly reviewed on the taxonomic riddles complicated in the genus *Metagonimus* in Japan and finally proposed 4 types of *Metagonimus* that have larger ventral sucker than the oral sucker; *Metagonimus* Yokogawa type (*M. yokogawai*), *Metagonimus* Miyata type, *Metagonimus* Koga type, and *Metagonimus* Takahashi type (*M. takahashii*). *Metagonimus* Miyata type was to designate the group of worms other than *M. yokogawai*, which encyst in *P. altivelis* or other kinds of fresh water fish except *C. auratus* and *C. carpio* and produce intermediate-sized eggs. *Metagonimus* Koga type was to call the worms which encyst only on the scale of the dace and produce large-sized eggs. *Metagonimus* Takahashi type (*M. takahashii*) was to designate the worms that encyst in *C. auratus* or *C. carpio* and produce large-sized eggs.

This new classification system looks quite plausible and was readily applicable especially for the worms agreed to the criteria of Miyata type. Many ambiguous worms described in the literature (Miyata, 1944; Kim, 1980), having some morphological difference from both *M. yokogawai* and *M. takahashii*, were well fitted to the criteria of Miyata type. Koga type was, however, hardly justifiable because morphologically it revealed not a single difference from *M. takahashii* (Saito, 1984). The only difference given by Saito (1984) was the kinds of their fish host and the site of encystment in the fish. For this reason, the present authors regarded Koga type to be a synonym of Takahashi type (*M. takahashii*).

Most of the present specimens of *Metagonimus*, originated from *T. taczanowskii*, were well compatible to newly proposed morphological types of *Metagonimus* (Saito, 1984), and it became clear that three types of *Metagonimus* are encysted in the dace caught from the Sumjin river, Korea. The specimens of *Metagonimus* Miyata type (Figs. 4 & 7) differed from *M. yokogawai* (Figs. 3 & 6) by posteriormost location of the right testis, separated left testis from the right one, distribution of uterine tubules over the left testis and intertesticular junction, no vitellaria distribution near the posterior end of the body,

and a little larger size of the eggs. The specimens diagnosed as *Metagonimus* Koga type (*M. takahashii*) (Figs. 5 & 8) differed from *M. yokogawai* or *Metagonimus* Miyata type by no posteriormost location of right testis, separated left testis from the right one, distribution of uterine tubules over the left testis and intertesticular junction, distribution of the vitellaria passing through the posteriormost portion of the body, and larger size of the eggs.

However, there were some points that should be mentioned. In the case of *Metagonimus* Miyata type, for example, many of the present specimens were easily classified as that, but there were not a few (70 undetermined) samples, either similar to *M. yokogawai* or to *M. takahashii* and hard to discriminate from them. It seems also worthwhile to note that among the specimens of *Metagonimus* collected from humans in Korea (Chai *et al.*, 1990), many were compatible to the criteria of Miyata type, however, some ambiguous specimens similar to *M. yokogawai* or *M. takahashii* were also encountered. This should mean that such classification system might not be suitable for all occasions and by no means a complete one. In fact, there is a possibility that *Metagonimus* Miyata type might be an intraspecific morphological variation of *M. yokogawai* or *M. takahashii*. In order to clarify this point and to nominate Miyata type as a new species of *Metagonimus*, as suggested by Saito (1984), whole life cycle studies from eggs to adults should be performed and its offsprings be proven to be unique and free from other types.

As for *M. takahashii*, the authors would like to accept its present taxonomic position, since it does have many unique features fairly differentiable from *M. yokogawai*. However, there also remained some unclear points especially the presence of intermediate forms between Miyata type and *M. takahashii*. Whole life cycle studies from eggs to adults, with observation of their offsprings, if possible, would also be very helpful for verification of the validity of *M. takahashii*.

Conclusively, the authors are of opinion that it should be further studied whether *Metagonimus* Miyata type and even *M. takahashii* are intraspecific morphological variations of *M. yokogawai*, or each distinctively different species.

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=국문초록=

섬진강산 황어에 피낭한 *Metagonimus* 속 흡충의 세 가지 형태학적 유형

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섬진강 유역(경남 하동군)산 황어(*Tribolodon taczanowskii*)로부터 *Metagonimus*속 피낭유충을 다수 검출하고 실험적으로 흰쥐와 햄스터에 감염시킨 후 소장으로부터 성충을 얻어 관찰한 바 Saito(1984)의 분류에 따라 *Metagonimus* Yokogawa형 (*M. yokogawai*), Miyata형 및 Koga형 등 3가지로 나눌 수 있었다.

실험감염 결과, 흰쥐 4마리 및 햄스터 4마리로부터 *Metagonimus* 충체 총 1,624마리를 회수하였고 그 중 Yokogawa형은 820마리, Miyata형은 688마리, Koga형은 46마리이었다. 나머지 충체 70마리는 유형을 결정하기 어려웠다. Yokogawa형과 Miyata형의 주요 감별점은 두 고환의 위치, 자궁관의 분포 및 충란의 크기 등이었고, Miyata형과 Koga형의 감별점은 두 고환의 위치, 난황선의 분포, 충란의 크기 등이었다. 이들 유형 특히 Miyata형의 별종 가능성에 대해서는 많은 증거가 얻어질 때까지 이를 유보해야 할 것으로 생각되었고, Koga형은 중간숙주의 차이 이외에는 형태학적으로 Takahashi형 (*M. takahashii*)과 전혀 차이가 인정되지 않으므로 이와 동일한 것으로 취급하는 것이 옳을 것으로 판단되었다.

이상의 결과로 섬진강산 황어에는 3가지 유형의 *Metagonimus*가 피낭하고 있음을 확인하였다.

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