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Classification and host specificity of *Metagonimus* spp. from Korean freshwater fish

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Abstract: Taxonomic problems of *Metagonimus* spp. in Korea were investigated. Metacercariae of various freshwater fish species — *Plecoglossus altivelis, Carassius auratus, Zacco platypus, Zacco temmincki, Opsariichthys bidens* — were collected from different localities in Korea and experimentally fed to golden hamsters. Observation of recovered adult worms showed that *Plecoglossus altivelis* was infected with metacercariae of both *M. yokogawai* and *M. takahashii*. *C. auratus* was infected with metacercariae of *M. takahashii* and *Z. platypus, Z. temmincki, O. bidens* were infected only with metacercariae of *Metagonimus* Miyata type. From the inferences about the morphological characteristics, host specificities and occurrence patterns in infected animals, *Metagonimus* Miyata type is considered to be an independent group.

Key words: *Metagonimus yokogawai, M. takahashii, Metagonimus* Miyata type, metacercariae, freshwater fish, infection experiments, taxonomy

INTRODUCTION

Metagonimiasis is one of the most prevailing helminthic diseases of humans in Korea. Human infections are acquired by eating raw freshwater fish. During the past several decades, most epidemiological studies of metagonimiasis in Korea have been conducted along rivers where *Plecoglossus altivelis* are found with only one species, *Metagonimus yokogawai* Katsurada, 1912, implicated as the disease causing agent (Kang *et al.*, 1964; Chai *et al.*, 1977; Soh and Ahn, 1978; Suh and Choi, 1979; Kim *et al.*, 1979; Seo *et al.*, 1981, 1982; Ahn, 1984; Song *et al.*, 1985; Ahn *et al.*, 1987; Sohn *et al.*, 1990 etc.). Recently, however, other species or types of *Metagonimus*, namely *M. takahashii* Suzuki, 1930 and *Metagonimus* Miyata type of Saito, 1984 were reported from various species of freshwater fish (Kim, 1980; Kim *et al.*, 1987; Ahn and Ryang, 1988; Chai *et al.*, 1993; Ahn, 1993; Yu *et al.*, 1994) or humans (Ahn and Ryang, 1988; Chai *et al.*, 1993). However, variations in adult morphology resulted in confusion with regard to the validity of these species.

In the present study, to resolve the taxonomic problems of *Metagonimus* spp. in Korea, metacercariae of various freshwater fish species were collected from different localities in Korea and experimentally fed to golden

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hamsters.

MATERIALS AND METHODS

1. Sampling localities of fish

During the period from March through October, 1995, fish *Plecoglossus altivelis*, *Carassius auratus*, *Zacco platypus*, *Zacco temmincki*, *Opsariichthys bidens* — were collected using cast-nets. Sampling localities are:

Site 1. Hyun-ri, Kapyong-gun, Kyonggi-do

Site 2. Osipchon (stream), Samchok-gun, Kangwon-do

Site 3. Choyanggang (river), Chongson-gun, Kangwon-do

Site 4. Koesan-gun, Chungchongbuk-do

Site 5. Kwangsi-myon, Yesan-gun, Chungchongnam-do

Site 6. Chong-yang-gun, Chungchongnam-do

Site 7. Puan-gun, Chollabuk-do

Site 8. Tongchon (stream), Kwang-yang-gun, Chollanam-do

Site 9. Posong-gun, Chollanam-do

Site 10. Kangjin-gun, Chollanam-do

Site 11. Punggi-up, Yechon-gun, Kyongsangbuk-do

Site 12. Chongdo-gun, Kyongsangbuk-do

Site 13. Kochang-gun, Kyongsangnam-do

Site 14. Sanyang-chon (stream), Koje-gun, Kyongsangnam-do

Site 15. Hwagae-myon, Hadong-gun, Kyongsangnam-do (purchased)

Site 16. Kyongdong Market, Seoul (purchased)

2. Animal infections

To minimize definitive host variation, all hamsters used for experimental infections were progeny derived from one parent.

Experimental infections were conducted using two methods: a) the metacercariae of *Metagonimus* were collected using artificial gastric juice and the hamsters infected orally. b) fish confirmed to be infected with metacercariae of *Metagonimus*, were fed to hamsters which were starved for one day.

3. Worm recovery and preparation

Hamsters infected with metacercariae of *Metagonimus* were sacrificed by cervical dislocation. The whole intestine was opened longitudinally in 0.85% saline and examined under a stereomicroscope. Recovered worms were not flattened to avoid variations by fixing pressure. The worms were directly fixed in hot AFA and stained with Semichon's acetocarmine.

RESULTS

1. Species composition of *Metagonimus* spp. infections

Three species, *Metagonimus yokogawai*, *M. takahashii*, and *Metagonimus* Miyata type, were obtained from the experimental infection of hamsters with *Metagonimus* metacercariae. The species composition, according to the infections of various 2nd intermediate hosts, is summarized in Table 1.

Hamsters fed *P. altivelis* caught at Osipchon, Samchok-gun and bought at Hwagae-myon became infected with *M. yokogawai* (one individual was unidentified). *P. altivelis* caught at Kwangyang were infected with both *M. yokogawai* and *M. takahashii*. Among the recovered worms, the number of *M. takahashii* was exceedingly larger than that of *M. yokogawai* (three individuals were unidentified).

From the infection experiments of *Carassius auratus*, except for one variation type, only *M. takahashii*, was recovered. All of the worms isolated from *Zacco platypus*, *Z. temincki* and *Opsariichthys bidens* were identified as *Metagonimus* Miyata type, regardless of collecting localities.

2. Adult morphology of *Metagonimus* spp. obtained from this study

The morphological characteristics of *Metagonimus yokogawai* Katsurada (1912) (Fig. 1), *M. takahashii* Suzuki, 1930 (Fig. 2) and *Metagonimus* Miyata type (Fig. 3) obtained from this study were well coincide with the descriptions of other authors (Chai *et al.*, 1993; Saito, 1984). Therefore the detailed description of each species was omitted in this study. The measurements of several charaters of each species are given in Table 2. The egg sizes of each species in this study were somewhat

Table 1. The species composition of recovered worms according to the species of fishes

Fish species	Sampling site	Date of infection	Duration of infection (days)	Number of worms observed	Individual number of			
					My	Mt	Mm	Va
P. altivelis	Site 2	940824	11	9	8	0	0	1 [a]
P. altivelis	Site 8	941010	18	36	2	31	0	3 [b]
P. altivelis	Site 15	941014	14	4	4	0	0	0
C. auratus	Site 8	941011	7	16	0	15	0	1 [c]
C. auratus	Site 7	941014	10	2	0	2	0	0
Z. platypus	Site 1	940323	12	11	0	0	11	0
Z. platypus	Site 5	940729	8	13	0	0	13	0
Z. platypus	Site 4	940801	16	72	0	0	72	0
Z. platypus	Site 13	940904	9	2	0	0	2	0
Z. platypus	Site 9	941007	21	4	0	0	4	0
Z. platypus	Site 10	941014	14	6	0	0	6	0
Z. temmincki	Site 3	940816	7	2	0	0	2	0
Z. temmincki	Site 12	940916	14	1	0	0	1	0
Z. temmincki	Site 14	940927	15	22	0	0	22	0
Z. temmincki	Site 11	940927	15	13	0	0	13	0
Z. temmincki	Site 9	941007	15	1	0	0	1	0
O. bidens	Site 6	940703	34	3	0	0	3	0
O. bidens	Site 16	940203	12	6	0	0	6	0

My, *Metagonimus yokogawai*; Mt, *Metagonimus takahashii*; Mm, *Metagonimus* Miyata type; Va, variation type. [a]variation type I; [b]variation type II; [c]variation type III. *P. altivelis*, *Plecoglossus altivelis*; *C. auratus*, *Carassius auratus*; *Z. platypus*, *Zacco platypus*; *Z. temmincki*, *Zacco temmincki*; *O. bidens*, *Opsariichthys bidens*.

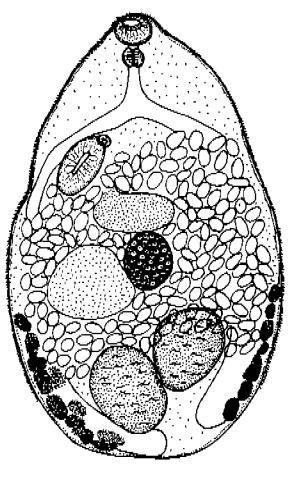

Fig. 1

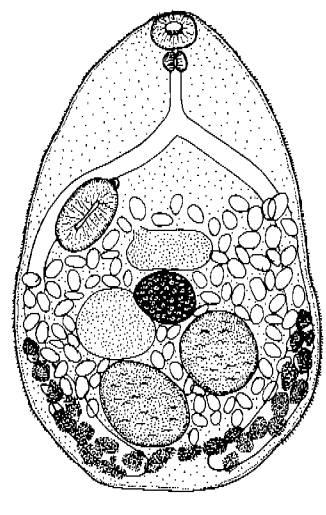

Fig. 2

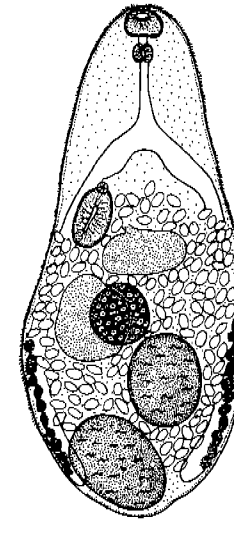

Fig. 3

Figs. 1-3. The adult morphology of three kinds of *Metagonimus* (ventral view) **1.** *Metagonimus yokogawai* from the metacercaria of *P. altivelis* **2.** *Metagonimus takahashii* from the metacercaria of *P. altivelis* **3.** *Metagonimus* Miyata type from the metacercaria of *Z. platypus* (Scale bar: 0.1 mm).

Table 2. Dimensions of *Metagonimus* spp. from the present study (based on the unflattened and stained specimens; width × length)

Measurement (mm)	*M. yokogawai* (10 individuals)	*M. takahashii* (10 individuals)	*M.* Miyata type (12 individuals)
Hosts	*P. altivelis*	*P. altivelis*, *C. auratus*	*Z. platypus*, *Z. temmincki* *O. bidens*
Length	0.51-0.71	0.65-0.74	0.41-0.93
Breadth	0.21-0.36	0.28-0.42	0.18-0.41
Oral sucker	0.040-0.068 x 0.035-0.048	0.055-0.070 x 0.040-0.057	0.048-0.065 x 0.037-0.053
Ventral sucker	0.032-0.063 x 0.065-0.128	0.053-0.068 x 0.090-0.115	0.040-0.083 x 0.068-0.120
Pharynx	0.020-0.038 x 0.027-0.038	0.032-0.042 x 0.033-0.048	0.025-0.040 x 0.030-0.040
Left testis	0.070-0.110 x 0.083-0.137	0.090-0.127 x 0.113-0.145	0.070-0.128 x 0.078-0.170
Right testis	0.065-0.105 x 0.102-0.165	0.090-0.123 x 0.110-0.160	0.070-0.148 x 0.095-0.185
Ovary	0.043-0.072 x 0.043-0.100	0.053-0.100 x 0.060-0.080	0.050-0.090 x 0.050-0.098
Eggs	0.015-0.018 x 0.024-0.028 (av. 0.017 x 0.027)	0.018-0.021 x 0.028-0.033 (av. 0.020 x 0.032)	0.014-0.019 x 0.025-0.030 (av. 0.019 x 0.029)

smaller than those in other reports bacause of measuring stained specimens.

3. Brief comments on each variations

Variation type I: The location of testes, the vitelline follicles distributions and the egg sizes are identical with *M. yokogawai*. But the left testis is surrounded by uterine coils.

Variation type II: All characters are in accord with the characters of *M. takahashii* except the distribution of uterine coils. Namely, the uterine coils do not cross the intertesticular junction like *M. yokogawai*.

Variation type III: Only the egg sizes are identical with *Metagonimus* Miyata type. The other characters are in agreement with *M. takahashii*.

DISCUSSION

Five species have hitherto been recognized in the genus *Metagonimus*. *Metagonimus yokogawai* (Katsurada, 1912), *M. takahashii* Suzuki, 1930, *M. minutus* Katsuta, 1932, *M. katsuradai* Izumi, 1935, and *M. otsurui* Saito and Shimizu, 1968. *M. yokogawai*, *M. takahashii* and *M. minutus* are easily distinguished from *M. katsuradai* and *M. otsurui* in that their oral sucker is smaller than the ventral sucker. *M. minutus* differs morphologically from *M. yokogawai* and *M. takahashii* by a smaller body and eggs. There have been no reports about *M. minutus* since it was described as a new species (Katsuta, 1932).

During the past several decades, many controversies over the taxonomic status of *M. takahashii* have been presented. Takahashi (1929) and Suzuki (1930) considered *M. takahashii* as an independent species since the eggs of *M. takahashii* were larger than eggs of *M. yokogawai* and the 2nd intermediate host of *M. takahashii* was not *P. altivelis*, but cyprinid fish. Many authors, however, considered *M. takahashii* as a synonym of *M. yokogawai*, because the only diagnostic character of these two species was the egg size (Asada, 1934; Ito, 1964). On the other hand, Morishita (1951) proposed to call *M. takahashii* as *M. yokogawai* var. *takahashii*. From the morphological comparisons of cercariae, metacercariae and adults of *M. yokogawai* and *M. takahashii*, Saito (1972) confirmed that both were independent species. Furthermore, Saito (1973) showed that *P. altivelis* was highly susceptible to cercariae of *M. yokogawai* whereas *C. auratus* was more susceptible to *M. takahashii*. Chai *et al.* (1993) evaluated *M. takahashii* as a distinct species, based on several morphological characteristics of adult worms as well as the peculiarly large size of their eggs.

In the present study, *M. takahashii* is treated as an independent species based on the several adult characteristics. The most conspicuous distinguishing character between these two

species is the distribution pattern of the uterine coils, principally, the uterine coils of *M. takahashii* cross the intertesticular junction whereas those of *M. yokogawai* extend to the anterior or mid region of the left testis. Usually the largest eggs of *M. takahashii* are larger than the largest eggs of *M. yokogawai* and this difference can be used as a major distinguishing character between these two species. However, the smallest eggs of *M. takahashii* often overlap with the largest eggs of *M. yokogawai*. Therefore it is occasionally difficult to distinguish either species from mixed populations based only on the measurements of a few eggs. In some adult *M. takahashii*, the intertesticular gap is not present, so this character can not be used as a diagnostic feature of these two species. From the results of experimental infections, the metacercariae of *M. takahashii* infect both *C. auratus* and *P. altivelis*. This is the first record that *P. altivelis* acts as a 2nd intermediate host of *M. takahashii* which can resolve some confusion about adults with large size eggs originating from *P. altivelis*.

Saito (1984) proposed four types of *Metagonimus* which have larger ventral sucker than oral sucker. Among those types, the most problematic type is *Metagonimus* Miyata type. In the present study, *Metagonimus* Miyata type is considered as an independent group.

Metagonimus Miyata type is distinguished from *M. yokogawai* by the intertesticular distribution of uterine coils, larger eggs, and the location of the right testis which extends beyond the terminal end of the caecum. *Metagonimus* Miyata type differs from *M. takahashii* by the posterior location of the right testis, smaller size of eggs, and no vitellaria distribution near the posterior end of the body.

The general morphology of *Metagonimus* Miyata type appears to be an intermediate form between *M. yokogawai* and *M. takahashii*. Because of this, some authors considered *Metagonimus* Miyata type as a hybrid of *M. yokogawai* and *M. takahashii* or as a variation type of these two species.

Most species of digeneans are hermaphroditic, and syngamy can occur between gametes from different individuals (amphimixis) or from a single individual (automixis). Studies have not been conducted on the fertilization type for species of *Metagonimus*. According to the reports about infection, single worm infections were not observed. Therefore the possibility of cross-fertilization can not be excluded. From this point of view, if definitive host is infected with both *M. yokogawai* and *M. takahashii*, cross-fertilization between individuals of these two species may occur, producing hybrid. Often hybrid zygotes are aborted soon after their formation, or at any stage of the life cycle. If a hybrid survives until adult, the diminished fitness may lead to hybrid breakdown (Dobzhansky *et al.*, 1977). The possibility that hybrids between *M. yokogawai* and *M. takahashii* are produced and form large independent populations, observed in many reports and the present study, is very low. Furthermore, the 2nd intermediate hosts of *M. yokogawai* are restricted to three species, *Plecoglossus altivelis*, *Tribolodon taczanowskii* and *Lateolabrax japonicus*, in Korea (Chai *et al.*, 1991; Ahn, 1983). From the results of the present experiments and other authors' (Chun, 1960; Chai *et al.*, 1991, 1993), the 2nd intermediate hosts of *M. takahashii* are *P. altivelis*, *T. taczanowskii* and *Carassius auratus*. Therefore, to produce hybrids, the definitive host must eat either *P. altivelis* or *T. taczanowskii*. Hybrid offsprings produced by cross-fertilization between these two species can not occupy the total population of recovered worms in infected animals. However, residents who live in areas where these two fish species are not caught and do not eat these two fish species are only infected with *Metagonimus* Miyata type. From this it can be concluded that *Metagonimus* Miyata type is not hybrid between *M. yokogawai* and *M. takahashii*.

The hypothesis that *Metagonimus* Miyata type is a variation of either *M. yokogawai* or *M. takahashii* can also be postulated. It is well known that the structural, physiological, and behavioral characteristics of a parasite population may be influenced by variations in host relationships (Haley, 1962). The studies about intraspecific variations related to the host species, however, were concentrated mainly on the variations in the different

definitive hosts. Intraspecific variations of digeneans according to the different 2nd intermediate hosts are not well documented. If the above hypothesis is cornect, the variations will be derived not from the definitive hosts but from the intermediate hosts. Then, the only way to explain the hypothesis is that some factors of the intermediate hosts may affect a gene or genes of the infected worms and the phenotypic differences of adult worms are the results of expression or suppression of such genes. On the other hand, if the above hypothesis is false, it can be concluded that the 2nd intermediate host specificities of each kinds of *Metagonimus* are very specific.

Generally, the principal basis for classifying helminth parasites is an analysis of adult morphology and host relationships. From the present observation and many other reports, the morphological features of *Metagonimus* Miyata type are clearly distinctive from the other species of *Metagonimus*. Furthermore, the 2nd intermediate host specificities considerably high. Therefore, in the present study, we considered *Metagonimus* Miyata type as an independent group. To decide certainly the taxonomic category of *Metagonimus* Miyata type, the susceptibility of each fish species to each cercariae kinds and differences in molecular biological characteristics must be investigated.

Chai *et al.* (1991) reported three types of *Metagonimus* obtained from experimentally infected rats and hamsters by feeding dace resulting in some specimens which could not be identified. In the present study, some individuals also could not be identified. These individuals are considered to be variation among either *M. yokogawai* or *M. takahashii*.

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=초록=

식이성 윤충류질환의 관리전략 수립을 위한 감염원의 역학 및 병원체의 생물학적 특성에 관한 조사연구 — 한국산 민물어류에 기생하는 *Metagonimus*속 피낭유충의 숙주특이성과 감염실험을 통한 성충의 분류

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한국인에 널리 유행하는 *Metagonimus*속 흡충류의 분류학적 문제점들을 해결하기 위해 다양한 지역에서 채집한 여러 종의 민물어류로 부터 *Metagonimus*속 피낭유충을 검출하여 햄스터에 감염실험을 하였다. 감염실험 결과 은어는 기존에 알려져 있던 *M. yokogawai*의 피낭유충 외에 *M. takahashii*의 피낭유충에도 감염되어 있는 것이 밝혀졌으며, 붕어에는 *M. takahashii*의 피낭유충만이 감염되어 있었다. 피라미, 끄리, 갈겨니등에서 검출된 *Metagonimus*속 피낭유충의 감염실험 결과, 이들 어종은 모두가 *Metagonimus* Miyata type의 피낭유충에만 감염되어 있는 것으로 나타났다. *Metagonimus* Miyata type의 성충은 여러 가지 특징 및 숙주특이성 등에 의해서 다른 두 종과 구별되었으며, 그 분류학적 위치에 대해서 몇 가지 가능성을 고찰하였다.

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