The Korean Journal of Parasitology
Vol. 35, No. 4, 223-232, December 1997

Metagonimus miyatai sp. nov. (Digenea: Heterophyidae), a new intestinal trematode transmitted by freshwater fishes in Japan and Korea

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Abstract: *Metagonimus miyatai* n. sp. (Digenea: Heterophyidae) is described based on adult flukes collected from experimental dogs and hamsters fed with metacercariae encysted in the sweetfish, *Plecoglossus altivelis*, dace, *Tribolodon hakonensis* and *T. taczanowskii*, common fat-minnow, *Morocco steindachneri*, pale chub, *Zacco platypus*, and dark chub, *Zacco temmincki*, and on those collected from naturally infected humans. The new species was morphologically compared with *M. yokogawai* and *M. takahashii* obtained from experimental animals fed with the sweetfish and the crucian carp, *Carassius carassius*, respectively. The uterine loops of *M. miyatai* reached near the posterior end of the body through the space between the two testes, whereas those of *M. yokogawai*, occupied only the space between the acetabulum and anterior border of two testes. This uterine tubule distribution was similar to that of another closely related species, *M. takahashii*. However, vitellaria of *M. miyatai* ended in front of the posterior end of the left testis, while those of *M. takahashii* reached the posterior end of the left testis and ran it over. By raising *M. miyatai* as a new species, differentiation of *M. yokogawai* and *M. takahashii* became very clear. A key to the species of the genus *Metagonimus* in the Far East has been proposed.

Key words: *Metagonimus miyatai* sp. nov., taxonomy, adult fluke, *M. yokogawai*, *M. takahashii*, metacercaria, freshwater fish, experimental infection (dog and hamster)

INTRODUCTION

Metagonimus yokogawai (Digenea: Heterophyidae) was first described by Katsurada (1912) as the type species of the genus *Metagonimus*. This fluke has been known to be the major agent for human metagonimiasis along with large and small streams in Japan and Korea, where the sweetfish, *Plecoglossus altivelis*, are available (Ito, 1964; Chai and Lee, 1990). Another species eliciting human metagonimiasis has been known to be *M. takahashii* (Asada *et al.*, 1957; Ahn and Ryang, 1988), which produces large-sized eggs and is transmitted by the crucian carp, *Carassius*

• Received 7 November 1997, accepted after revision 26 November 1997.

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carassius (Takahashi, 1929).

In Korea, however, Kim (1980) reported a peculiar finding that the *Metagonimus* eggs and worms recovered from the people along the Keum River, where sweetfish are not available, were similar to but not identifiable with *M. yokogawai* or *M. takahashii*. In the meantime, one of the present authors (Saito, 1984) proposed to classify *Metagonimus* flukes, distributing in Japan, having larger ventral sucker compared with the oral sucker, into 4 types; Yokogawa type (= *M. yokogawai*), Miyata type, Koga type, and Takahashi type (= *M. takahashii*). The Miyata type was based on one of the two kinds of *Metagonimus* flukes encysted in *P. altivelis*, both of which were described under the name of *M. yokogawai* (Katsurada, 1912). This type also occurs in various kinds of freshwater fishes including *Tribolodon hakonensis*, *Zacco platypus*, and *Z. temmincki* (Saito, 1984).

The existence as well as taxonomic significance of the Miyata type was fully agreed in Korea by Kim *et al.* (1987), Chai *et al.* (1991 & 1993), and Rim *et al.* (1996). Furthermore, at the 2nd Japan-Korea Parasitologists' Seminar in April, 1996, significant results of morphological, biological, and genetic analyses of Yokogawa, Miyata, and Takahashi types were presented by several workers, and eventually it was concurred to consider the Miyata type as a distinct species.

Here, the present authors propose to designate *Metagonimus* Miyata type as a new species, *Metagonimus miyatai*. The name 'miyatai' is in dedication to the late Dr. Itoku Miyata, who first pointed out the existence of this type of *Metagonimus* flukes (Miyata, 1941 & 1944).

MATERIALS AND METHODS

Collection and morphological observation of metacercariae

The metacercariae of three species of *Metagonimus* were collected from the sweetfish, *P. altivelis*, dace, *T. hakonensis* and *T. taczanowskii*, pale chub, *Z. platypus*, dark chub, *Z. temmincki*, common fat-minnow, *Morocco steindachneri*, and crucian carp, *C. carassius*.

The sweetfish were collected from the Chikugo River (Hita City, Ohita Prefecture), the Ohta River (Hiroshima City, Hiroshima Prefecture), the Oguni River (Funagata Town, Yamagata Prefecture), and the Natori River (Sendai City, Miyagi Prefecture), Japan. The daces were caught from the Ohta River, the Daimon River (Yamagata City), Japan, and from the Sumjin River (Hadong-gun), Korea. The pale chubs, and dark chubs were caught from the Ohta River, Japan, the Namhan River (Umsong-gun) and several other localities, Korea, and common fat-minnows from the Daimon River, Japan. The crucian carps were collected from the Ohta River, the Lake Yoroigata, Japan, and the Namhan River, Korea.

From the retrospective view of experimental infection of dogs and hamsters, it was confirmed that most of the metacercariae of *M. miyatai* occur under the scale of the sweetfish, dace, pale chub, dark chub, and common fat-minnow. Whereas, metacercariae of *M. yokogawai* were found in the muscle of the sweetfish and dace, and those of *M. takahashii* under the scale of the crucian carp. Therefore, the metacercariae encysted in the muscle of *P. altivelis* (*M. yokogawai*), those under the scale of *P. altivelis* and *Z. platypus* (*M. miyatai*) and under the scale of *C. carassius* (*M. takahashii*) were used for morphological study of each species. The metacercariae were liberated from the tissue after incubation in artificial gastric juice consisting of 5 mg/ml pepsin (1:10,000) and 0.08 N HCl at 37°C for 1 to 2 hrs, and the size of the cyst was measured.

Excysted metacercariae were obtained by treatment with commercial intestinal juice "Trypsilin (Mochida Pharmaceutical Co., LTD.)" at room temperature for 10-20 min or by applying slight pressure on the cover slip. The excysted metacercariae were washed with saline, fixed in Schaudinn's solution under cover slip pressure, stained with Haiden-hain's iron-hematoxylin, acetocarmine, borax carmine, or PAS, and mounted in balsam.

Experimental infection of dogs and hamsters, and recovery of adult flukes

Domestic dogs and syrian golden hamsters were given pieces of fish tissues harboring metacercariae, and sacrificed 13-19 days post-

feeding. The small intestine of the infected animals was vertically opened with scissors, and rinsed with 0.9% NaCl. The adult flukes contained in the contents were washed with saline and collected under a dissecting microscope, fixed in 10% formalin or Schaudinn's solution under cover slip pressure, stained with acetocarmine or borax carmine, and mounted in balsam.

Recovery of *M. miyatai* adult flukes from naturally infected humans

Twenty-seven inhabitants residing in Yongweol-gun, Kangwon-do, Korea, who were positive for *Metagonimus* eggs bigger than those of *M. yokogawai*, were treated with praziquantel 10 mg/kg single dose and purged with 30 g $MgSO_4$ one hour later. The whole diarrheic stools were collected for 4-5 hrs, washed several times carefully with tap water, and observed under a dissecting microscope. Thousands of adult flukes were recovered, and some of them were fixed in 10% formalin under cover slip pressure, and stained with Semichon's acetocarmine for identification.

All measurements were given in μm unless otherwise stated. All figures were drawn with the aid of a camera lucida.

RESULTS

1. Description of *Metagonimus miyatai* n. sp.

Adults (Tables 1-2, Figs. 1-3): Morphology of adult flukes recovered from humans (Korea), experimental dogs (Japan), and hamsters

Table 1. Comparative measurements[a] of the adults of three species of the genus *Metagonimus* from experimental dogs and hamsters

Species	*M. miyatai* sp. nov.			*M. yokogawai*	*M. takahashii*
Fishes MC[b] obtained	*P. altivelis*	*T. taczanowskii*	*Z. temmincki*	*P. altivelis*	*C. carassius*
Locality	Oguni River Yamagata	Sumjin River Hadong-gun	Ohta River Hiroshima	Ohta River Hiroshima	Ohta River Hiroshima
Body[c]	998–1300 × 462–627	787–956 × 378–410	867–1137 × 399–459	800–1320 × 419–536	863–1193 × 444–571
Oral sucker	51–65 × 66–93	45–50 × 60–72	41–51 × 61–72	39–56 × 61–75	38–57 × 65–81
Pharynx	37–60 × 30–51	34–46 ×24–30	36–51 × 32–42	38–49 × 33–37	41–51 × 25–44
Acetabulum	98–131 × 74–90	90–102 × 63–75	85–109 × 65–77	89–118 × 65–79	96–114 × 66–79
Seminal vesicle	61–173 × 123–418	45–80 × 120–225	58–138 × 157–278	58–86 × 179–303	43–129 × 129–279
Seminal receptacle	113–355 × 74–254	74–180 × 75–190	88–189 × 63–242	159–272 × 99–264	61–145 × 124–312
Ovary	107–146 × 86–127	80–102 × 79–103	84–113 × 79–107	91–132 × 61–90	67–124 × 75–123
Left testis	228–302 × 173–230	155–251 × 146–193	176–250 × 146–188	162–256 × 117–187	180–256 × 127–178
Right testis	251–419 × 183–254	208–263 × 126–205	206–273 × 168–213	168–263 × 124–189	211–286 × 148–217

[a] μm, length × width; [b] metacercaria; [c] 10 specimens each were measured for each group.

Table 2. Comparative measurements[a] of the eggs of three species of the genus *Metagonimus* from the feces of experimentally infected dogs and hamsters

Species	Host for MC		Locality	No. eggs measured	Range (mean) of	
	Fishes	Parts			Length	Width
M. miyatai sp. nov.	*P. altivelis*	scale	Oguni River	36	28.2–31.6 (29.5)	15.8–18.4 (17.2)
	P. altivelis	scale	Natori River	35	27.4–31.6 (29.2)	16.1–19.2 (17.3)
	M. steindachneri	scale	Daimon River	32	26.3–30.5 (29.0)	15.8–17.0 (17.1)
	Z. temmincki	scale	Ohta River	31	26.6–32.1 (29.4)	16.8–18.9 (17.8)
	Z. platypus	scale	Namhan River	30	29.4–32.3 (29.6)	14.1–19.2 (17.1)
M. yokogawai	*P. altivelis*	muscle	Ohta River	35	26.1–30.3 (27.9)	15.5–18.2 (16.4)
	P. altivelis	muscle	Natori River	38	25.8–30.5 (27.4)	15.0–18.2 (16.0)
	T. hakonensis	scale	Ohta River	35	25.0–29.4 (27.7)	14.7–16.6 (15.8)
	P. altivelis	muscle	Sumjin River	30	25.6–29.4 (27.5)	14.1–17.9 (15.5)
M. takahashii	*C. carassius*	scale	Yoroi Lake	100	29.4–35.1 (32.4)	18.6–22.3 (20.1)
	C. carassius	scale	Ohta River	100	28.8–34.0 (31.8)	17.8–21.5 (19.7)
	C. auratus	scale	Namhan River	30	29.7–36.1 (32.8)	17.9–21.5 (19.8)

[a] μm.

(Korea) were identical, and their measurements were similar to one another. Therefore, description was based on 10 adult flukes recovered from dogs 14-17 days post-feeding of metacercariae isolated from *P. altivelis*, *Z. platypus*, *Z. temmincki*, and *M. steindachneri*.

Body flat and oval, tapering anteriorly; covered with numerous fairly conspicious scale-form spines on anterior half, and the number of spines gradually decreased posteriorly. Size of body 867-1300 × 399-627. Oral sucker subterminal, transversely elliptical, 41-65 × 61-93. Acetabulum 85-131 × 65-90, larger than oral sucker, located in right submedian line at almost one fourth of body length from anterior end and opened into genital atrium. Prepharynx short, followed by a well developed elliptical or pyriform pharynx, 36-60 × 24-51. Esophagus 49-120 long, bifurcated into ceca. Left cecum terminated blindly posterior to left testes, and right cecum ended in front of anterior two thirds of right testis with a few exceptions. Testes elliptical, located posterior half of body, lay obliquely tandem but separated by uterine loop one from the other. Right testis situated very close to lateral and posteriormost side of body. Vitellaria and cecum extending along right lateral side of body terminated in front of anterior two thirds of right testis. Left testis 176-302 × 146-230; right testis 206-419 × 126-254. Seminal vesicle, 51-173 × 123-418, situated in median line, sinister to acetabulum, and often divided into two parts by a constriction. Seminal receptacle irregularly elliptical, 88-355 × 63-293, situated dexter to ovary. The size of seminal vesicle and/or seminal receptacle remarkably varied according to the quantity of sperm contained. Ovary elliptical, equatorial, 84-146 × 86-127. Uterus, occupying all available space in hind body to near posterior end of body. Uterine loop passed between testes. Vitellaria occupied the region from level of ovary to posterior end of body. Dextral vitellaria extending along right lateral side of body, ended posteriorly in front of the point where right testis closely approached lateral side of body.

Uterine eggs ovoid, 26.3-32.1 (av. 29.4) × 15.8-19.2 (av. 17.4), light yellowish brown in color. Egg shell smooth, operculated at one end and had a very small blunt point at the other. Eggs embryonated. Excretory vesicle irregularly Y-formed, arms reaching anteriorly to ovary, pore at posterior end of body. Excretory vesicle contained small excretory granules.

Metacercariae (Table 3, Figs. 7 & 9): The cyst was flat, oval or elliptical in shape, and 153-180 × 140-158. The general morphology of the excysted metacercaria was closely similar to that of *M. yokogawai* and *M. takahashii*

Table 3. Comparative measurements[a] of the metacercariae of three species of the genus *Metagonimus*

Species	*M. miyatai* sp. nov.		*M. yokogawai*	*M. takahashii*
No. specimens measured	20	20	10	10
Fish	*M. steindachneri*	*Z. platypus*	*P. altivelis*	*C. carassius*
Locality	Diamon River Yamagata	Namhan River Umsong-gun	Ohta River Hiroshima	Yoroi Lake Niigata
Cyst	153–180 × 140–158	150–175 × 130–155	144–176 × 128–170	148–165 × 143–155
Body	335–490 × 145–200	310–402 × 140–185	300–435 x 150–195	340–395 × 117–145
Oral sucker	46–55 × 53–65	35–50 × 48–62	39–49 x 47–61	34–51 × 44–61
Pharynx	24–29 × 23–33	25–30 × 25–35	27–33 × 30–38	24–30 × 29–29
Acetabulum	27–32 × 32–39	27–36 × 7–36	27–36 × 27–30	28–42 × 27–29
Ovary	25–41 × 20–33	15–25 × 15–25	14–22 × 15–19	13–27 × 11–20
Left testis	18–39 × 16–30	18–32 × 14–25	18–32 × 13–22	16–25 × 11–18
Right testis	23–43 × 20–33	20–35 × 20–30	19–38 × 11–18	18–28 × 14–22

[a] μm, length × width.

(Takahashi, 1929; Komiya, 1965; Saito, 1972). The metacercaria was 335-490 × 145-200 in size, containing numerous yellow brownish granules in the body, and the uterine anlage ran between the two testes. The flame cell formula was 2 [(3+3+3) + (3+3+3)] = 36.

2. Taxonomic summary

Natural final hosts: man (Shimane Prefecture, May 17, 1984; Kochi Prefecture, Jun. 4, 1984; Kangwon-do, Jul. 10, 1991); dog (Yamagata City, Aug. 25, 1984); red fox, *Vulpes vulpes japonica* (Kaminoyama City, Yamagata Prefecture, Feb. 7, 1984; Yamagata City, Feb. 14, 1986); racoon dog, *Nyctereutes procyonoides viverrinus* (Yamagata City, Apr. 10, 1984; Apr. 13, 1985; Kaminoyama City, Yamagata Prefecture, Dec. 12, 1984; Dec. 12, 1985); black-eared kite, *Milvus migrans lineatus* (Higashine City, Yamagata Prefecture, Jul. 5, 1985).

Experimental host: mouse, rat, hamster, and dog (type host).

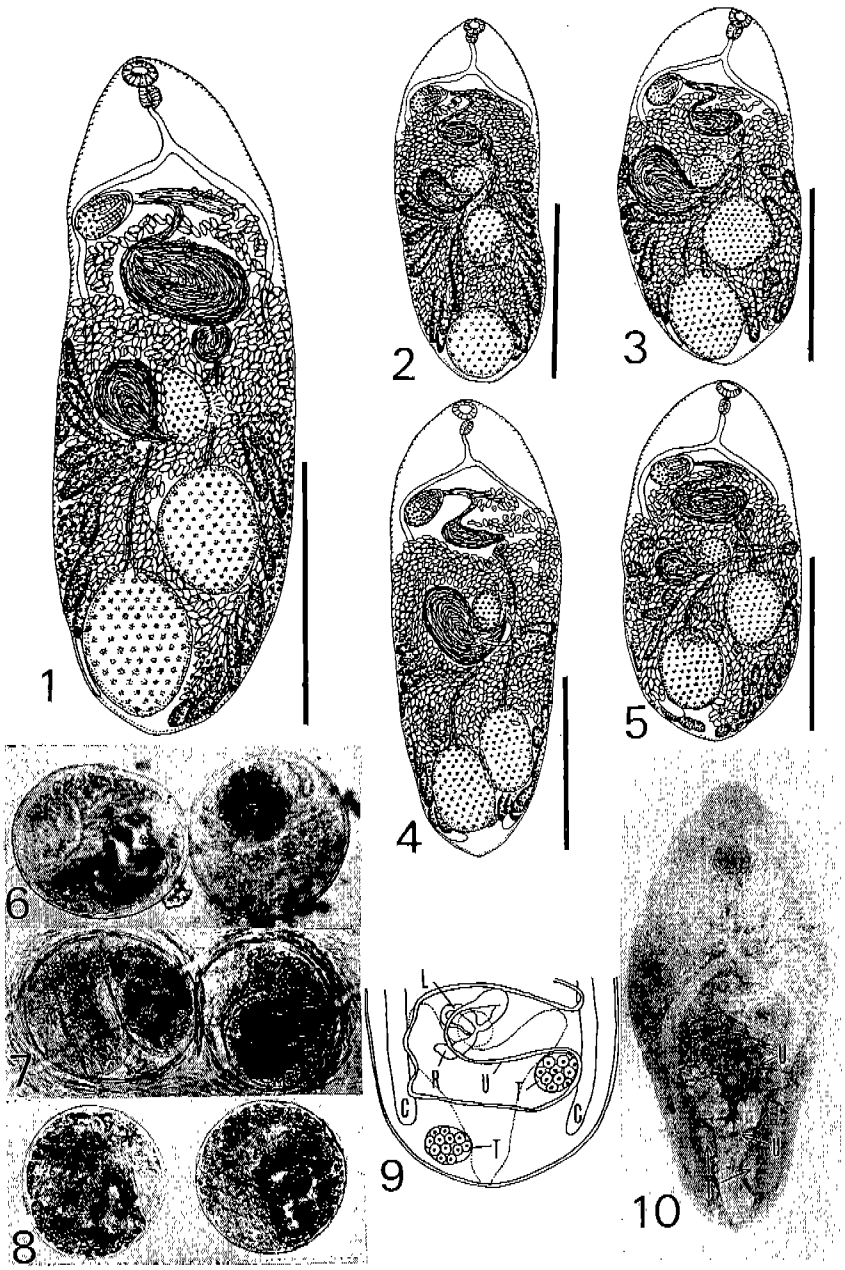
Habitats: small intestine, especially the lower part of the small intestine.

Type depository: Holotype and 14 paratypes are deposited in Department of Immunology and Parasitology, Yamagata University School of Medicine, Yamagata City, Japan. Fifty paratypes each are deposited in Department of Parasitology, Seoul National University College of Medicine, Seoul, Korea, and in Department of Fish Pathology, College of Fisheries Sciences, Pukyong National University, Pusan, Korea.

Second intermediate hosts: *Plecoglossus altivelis, Tribolodon hakonensis, Morocco steindachneri, Zacco platypus, Z. temmincki* (Japan); *T. taczanowskii, Opsariichthys bidens, Z. platypus, Z. temmincki* (Korea).

Sites of encystment: scale, fin, and head.

Geographical distribution: Iwate,



Figs. 1-3. Adults of *M. miyatai* n. sp. from dogs about two weeks post-feeding of metacercariae. **Fig. 1.** A holotype originating from the scale of *P. altivelis* in the Oguni River, Funagata Town, Yamagata Prefecture. **Fig. 2.** A paratype from *Z. temmincki* caught in the Ohta River, Hiroshima City. **Fig. 3.** A paratype from *M. steindachneri* from the Daimon River, Yamagata City. **Fig. 4.** An adult of *M. yokogawai* from a dog 16 days post-feeding of metacercariae encysted in the muscle of *P. altivelis* from the Ohta River. **Fig. 5.** An adult of *M. takahashii* from a dog 13 days post-feeding of the metacercariae encysted under the scale of *Z. temmincki* from the Ohta River. **Fig. 6.** Metacercariae of *M. yokogawai* from *P. altivelis*. **Fig. 7.** Metacercariae of *M. miyatai from M. steindachneri*. **Fig. 8.** Metacercariae of *M. takahashii* from *C. carassius*. **Fig. 9.** Posterior part of an excysted metacercaria of *M. miyatai*. C, caecum; L, Laurer's canal; R, seminal receptacle; T, testis; U, uterus. **Fig. 10.** An excysted metacercaria of *M. yokogawai* stained with PAS (dorsal view). Bar indicates 500 μm.

Yamagata (type locality), Miyagi, Niigata, Hiroshima, Kochi, and Ohita Prefectures, probably the whole of Japan. Inland areas of Kyonggi-do, Kangwon-do, Chungchongbuk-do, Chungchongnam-do, Chollanam-do, Chollabuk-do, Kyongsangbuk-do, and Kyongsangnam-do, Korea.

3. Remarks

The acetabulum of this new species is larger than the oral sucker, remarkably differing from the two known species, *M. katsuradai* and *M. otsurui*. The egg size of *M. minutus* is very small (23 × 13 μm) compared to other species of the genus *Metagonimus*. The distribution pattern of the uterine tubule of the new species is different from that of *M. yokogawai*, but similar to that of *M. takahashii*; uterine tubule of the new species (Figs. 1-3) reached near the posterior end of the body through the space between the two testes, whereas that of *M. yokogawai* occupied the space from the acetabulum to anterior border of the testes (Fig. 4). However, the vitellaria of the new species ended in front of the posterior end of the left testis, while that of *M. takahashii* reached the posterior end of the left testis or ran it over (Fig. 5).

Testes of *M. yokogawai* were situated side by side in contact with each other (Fig. 4). On the other hand, the testes of *M. miyatai* and *M. takahashii* were arranged obliquely tandem and apart from each other (Figs. 1-3 & 5). It was characteristic of *M. miyatai* that the right testis reached very close to the lateral and posteriormost side of the body. Eggs of the three species were different in their average length and width; *M. takahashii* was the largest, *M. yokogawai* the smallest, and *M. miyatai* in between (Table 2). However, the range of the egg size overlapped one another, so that the egg size itself can not be used as an absolute indicator for identification.

The metacercariae of *M. miyatai* from the scales of *P. altivelis* contained fine yellow brownish pigment granules in the body, particularly in the area along the esophagus and ceca, while those of *M. yokogawai* from the muscle of *P. altivelis* contained almost no granules (Figs. 6 & 7). Furthermore, the metacercariae of *M. takahashii* contained many coarse brownish pigment granules throughout the whole body (Fig. 8). The uterine anlage of the metacercaria of *M. yokogawai* occupied the space from the acetabulum to the anterior border of the testes (Fig. 10), but that of *M. miyatai* and *M. takahashii* metacercariae ran between the testes reaching near the posterior end of the body (Fig. 9). The uterine anlage was stained more strongly with Haidenhain's iron-hematoxylin than with borax carmine. Moreover, the uterine anlage of the metacercaria of *M. yokogawai* was stained very clearly with PAS (Fig. 10), though that of the metacercariae of *M. miyatai* and *M. takahashii* was stained weakly. The measured values of the three species were almost the same (Table 3).

DISCUSSION

In the genus *Metagonimus* Katsurada, 1912, five species have so far been described among the literature in the Far East; *M. yokogawai* (Katsurada, 1912), *M. takahashii* Suzuki, 1930, *M. minutus* Katsuda, 1932, *M. katsuradai* Izumi, 1935, and *M. otsurui* Saito *et* Shimizu, 1968. We add here *M. miyatai* n. sp. as the 6th species. Actually, however, *M. miyatai* was first found by Katsurada (1912), together with *M. yokogawai*. He (Katsurada, 1912) provided two photographs of adult worms (his Figs. 2 and 3) for *M. yokogawai*, but one of them is now morphologically identical with *M. miyatai*. The uterus in his Fig. 2 occupies the space from the acetabulum to the anterior border of the testes, consistent with *M. yokogawai*, but in his Fig. 3 it passes through the intertesticular junction, agreeing to *M. miyatai*.

Therefore, it became clear that *M. yokogawai* described by Katsurada (1912) was conspecific and should be divided into two species. The adult fluke in his Fig. 2 should be considered as the true sense of "*M. yokogawai*" and that in his Fig. 3 (obtained from a man) has to be designated as *M. miyatai*. The specimen reported under the name of *Loxotrema ovatum* (Kobayashi, 1912), a synonym of *M. yokogawai*, was the true sense of "*M. yokogawai*" viewing from the figure.

During the past seventy years, the discrimi-

nation of *M. yokogawai*, *M. takahashii*, and other related species has been discussed by many Japanese parasitologists, with many debates (Takahashi, 1929; Asada, 1934; Koga, 1938; Kogame, 1939; Miyata, 1941 & 1944; Takabayashi, 1953; Ochi, 1957; Komiya *et al.*, 1958; Ishii *et al.*, 1959; Saito, 1968a, 1968b & 1972; Saito and Yamashita, 1982). With the description of *M. miyatai* sp. nov., however, it became clear and easy to discriminate the species of the genus *Metagonimus* occurring in the Far East, according to the following key.

Key to the species of the genus *Metagonimus* in the Far East.

1. Acetabulum larger than oral sucker
---------- 2

Acetabulum smaller than oral sucker
---------- 5

2. Uterus occupying the space from acetabulum to anterior border of testes---------- 3

Uterus running between testes, and reaching near posterior end of body---------- 4

3. Eggs 26-30 μm in length
---------- *M. yokogawai*

Eggs 21-24 μm in length
---------- *M. minutus*

4. Vitellaria extending along right lateral side of body and mostly ending in front of anterior two thirds of right testis; eggs 29-30 μm in average length; parasitic in lower part of small intestine
---------- *M. miyatai* sp. nov.

Vitellaria extending along right lateral side of body reaching or running over posterior end of right testis; eggs 31-33 μm in average length; parasitic in upper part of small intestine
---------- *M. takahashii*

5. Seminal receptacle located on right side of ovary between seminal vesicle and left testis
---------- *M. otsurui*

Seminal receptacle located on left side of ovary between seminal vesicle and left testis
---------- *M. katsuradai*

Using the above key, the adult flukes originating from whitebaite, *Salanx microdon* (Mori, 1935; Komiya *et al.*, 1958; Suzuki *et al.*, 1963; Shimizu, 1970) and the skin of *T. hakonensis* (Koga, 1938; Kogame, 1939; Saito, 1968a; Yoshimura *et al.*, 1972) in Japan may be identified as *M. takahashii* and/or *M. miyatai*. Also the adults of *M. romanicum*, *M. parvus*, *etc.*, which are distributing in Europe (Ciurea 1915; 1924), are similar to *M. takahashii* except for Figs. 10 and 11 in Ciurea's paper (1924), which are considered to be adults of *M. yokogawai*.

All of adult flukes reported as the Miyata type in the Korean literature (Kim *et al.*, 1987; Chai *et al.*, 1991 & 1993; Ahn, 1993; Rim *et al.*, 1996) should be considered those of *M. miyatai*.

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