

***Metagonimus otsurui* Metacercarial Infection in a Gobiid Fish
(*Tridentiger brevispinis*) Collected from Lake Ogawara
in Aomori Prefecture, Japan**

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Abstract

From August 1989 to July 1995, a total of 1,127 gobiid fish, *Tridentiger brevispinis*, were collected in Lake Ogawara in Aomori Prefecture, and were examined for the prevalence of metacercarial (Mc) infection. Only *Metagonimus otsurui* Mc were found in the fish. A total of 829 of 1,067 (77.7%) fish were infected with the Mc. Each infected fish harbored 1 to 301 Mc (mean: 14.9), and a total of 12,347 Mc were recovered. As the body length of fish increased, the intensity of Mc also increased. More than half of the Mc (52.3%) were found in the fins, however, Mc were also recovered from several regions of the fish such as scales, epidermis, gills, head, visceral organs and muscles. One thousand and seven adult flukes recovered from the small intestine of 8 golden hamsters, which were used as an experimental final host, were all identified as *M. otsurui*. The present report is the first record that *M. otsurui* Mc was found in *T. brevispinis* in Japan. This survey was conducted to confirm the geographical distribution of *M. otsurui* in Aomori Prefecture, Japan.

Key words: *Metagonimus otsurui*; metacercaria; goby; *Tridentiger brevispinis*; survey.

Introduction

Trematodes of the genus *Metagonimus* are found in the small intestine of various mammals and birds. Five species of *Metagonimus*, *M. yokogawai* (Katsurada, 1912), *M. takahashii* (Takahashi, 1929), *M. katsuradai* (Izumi, 1935), *M. minutus* (Katsuta, 1932) and *M. otsurui* (Saito and Shimizu, 1968) have been reported as native to Japan. Metacercarial infection with the *Metagonimus* species was found in various species of freshwater fish, which were recognized as the second intermediate hosts in the life cycles of the trematodes (Ito, 1964; Komiya, 1965).

Metacercariae of *M. otsurui* were first found in three species of gobiid freshwater fishes, *Tridentiger obscurus*, *Chaenogobius castane* and *Chaenogobius urotaenia* collected at Lake Kasumigaura, Ibaraki Prefecture, Japan (Saito and Shimizu, 1968). They

were proposed as new species based on the detailed morphological features of the adult flukes which were obtained from golden hamsters. However, detailed knowledge concerning the life cycle of the parasite is not well known, even when golden hamsters and rats were successful as the final hosts of *M. otsurui* (Saito *et al.*, 1969).

In our previous surveys (unpublished data), a few flukes of *Metagonimus otsurui*-like species were found in the small intestine of *Rattus norvegicus*, which were collected at around areas of Lake Ogawara in Aomori Prefecture. Therefore, we made a survey of a gobiid fish, *T. brevispinis* for the prevalence of *M. otsurui* Mc infection.

Materials and Methods

From August 1989 to July 1995, 1,127 *T. brevispinis* (4.0–9.5 cm in body length) were collected from 2 localities (Sakaenuma and Asahi-cho) around Lake Ogawara in Aomori Prefecture, Japan. Each fish was weighed and measured before dissection.

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Detection of metacercariae (Mc)

All of the 1,067 fish were cut into small pieces, and digested individually in an artificial gastric juice (distilled water 1,000 ml, pepsin 5 g, 36% HCl 5 ml) at 37°C for 40–50 minutes. To determine the location of Mc in the fish body, 150 fish were divided into 7 parts, i.e., head, gills, fins, scales, epidermis, visceral organs and muscle. After precipitation of the digested homogenate for about 15 minutes at room temperature, the sediments were washed 4 times with physiological saline, and then examined under a dissecting microscope. Encysted Mc were measured under the light microscope with a micrometer. To collect excysted Mc, the encysted Mc were put into artificial intestinal juice (distilled water 100 ml, trypsin 1 g, NaHCO₃ 0.04 g, dog bile 1 ml) at 37°C for 15–30 minutes. The living excysted Mc were observed under slight pressure of a small cover glass.

Experimental infection to animals

Eight golden hamsters (Std: Syrian, males, 4–5 wks old) were used as the experimental final host. Two hundred encysted Mc were administered orally to each animal. Then the animals were bled to death on the 15th day after administration under general anesthesia using ether. The complete digestive tract was examined for fluke infection using a dissecting microscope.

Flukes and eggs

The recovered flukes were fixed with 70% alcohol under pressure between a slide glass and a cover glass, and stained with Borax-carmin, cleared in

xylene and mounted in Canada-balsam. They were used for the morphological observations and measurements. The produced eggs were also observed after being preserved in 10% formalin.

Results

The prevalence and intensity of Metagonimus Mc in fish

In this survey, only single-formed Mc were detected. A total of 829 fish (616 collected from Sakaenuma and 213 from Asahi-cho around Lake Ogawara) were infected with *Metagonimus* Mc. A total of 12,347 Mc were obtained from all of the fish. The mean infection rate in the two localities was 77.7%. The number of Mc per positive fish ranged from 1 to 301 (mean: 14.9). The relationship between the body length of the 1,067 fish and the prevalence of Mc is summarized in Table 1. The Mc were found in fishes longer than 3.5 cm in body length. As the body length increased, the mean number of Mc per fish also increased. The infection rate, however, decreased. Some of the Mc infected fishes showed blackish spots on their epidermis.

Recovery site of Mc

The location of Mc in the fish is shown in Table 2. The recovery rate in each part was different, ranging from 0.2 to 52.3%. Many Mc were found in the fins, epidermis, scales, and head, and the recovery rates were 52.3%, 16.2%, 14.3%, and 13.2%, respectively.

Table 1 Relationship between the body length of fish and number of *Metagonimus otsurui* metacercariae (Mc)

Body length range (cm) of fishes	No. of fishes		Prevalence (%)	No. of Mc recovered	No. of Mc per positive fish
	examined	positive			
≤4.9	63	63	100	328	5.2
5.0–5.9	258	246	95.3	1,576	6.4
6.0–6.9	313	250	79.9	3,526	14.1
7.0–7.9	319	211	66.1	5,073	24.0
8.0≤	114	59	51.8	1,844	31.3
Total	1,067	829	77.7	12,347	14.9

Table 2 Recovery sites of *Metagonimus otsurui* metacercariae (Mc) in 150 *Tridentiger brevispinis*

No. (%) of Mc recovered from							Total
fins	scales	epidermis	gills	head	viscera	muscles	
671 (52.3)	184 (14.3)	208 (16.2)	25 (2.0)	169 (13.2)	2 (0.2)	25 (2.0)	1,284

Morphology of Mc

The encysted Mc were yellowish in color, spherical in shape and measured $156\text{--}188 \times 130\text{--}184 \mu\text{m}$ in size (Fig. 1). The capsules enveloping the larvae consisted of two layers, a thick outer cyst wall and a thin inner one, measuring $8\text{--}18 \mu\text{m}$ in thickness. Excysted Mc were flat and elliptical in shape. The excysted Mc body was yellowish or brownish in color, and measured $380\text{--}632 \mu\text{m}$ in length and $152\text{--}248 \mu\text{m}$ in width. The body surface was covered with minute single spines. The oral sucker was located ventrally at the anterior subterminal end, elliptical in shape, and measured $30\text{--}62 \times 44\text{--}70 \mu\text{m}$ in size. The acetabulum was located at the anterior third of the body, was almost elliptical in shape, and

measured $32\text{--}52 \times 26\text{--}56 \mu\text{m}$ in size. Reproductive organs were immature. The testicular and ovarian primordia were situated behind the acetabulum. The excretory vesicle was irregularly Y-formed, and found in the median portion of the posterior end of the body.

Recovery of adult flukes

Eight golden hamsters were infected with *Metagonimus* flukes. As shown in Table 3, a total of 1,007 flukes; 2 (0.2%), 314 (31.2%) and 691 (68.6%), were recovered from the upper, middle and lower portions of the small intestine, respectively. The recovery rates ranged from 44.5% to 87.5% (mean: 62.9%). All flukes were sexually mature with many eggs in their uteri (Fig. 2).

Morphology of adult flukes and their eggs

The adult flukes were flat, elliptically elongated in shape, and measured $424\text{--}808 \mu\text{m}$ in length and $192\text{--}320 \mu\text{m}$ in width. The surface of the adult flukes were covered with scale-formed spines in the anterior half of the body. The oral sucker was transversely elliptical, and measured $28\text{--}48 \times 40\text{--}60 \mu\text{m}$ in size. The acetabulum, opening to the genital atrium, was situated to the right of the median line in the anterior third of the body length. It was elliptical in shape and measured $26\text{--}76 \mu\text{m}$ in length and $24\text{--}56 \mu\text{m}$ in width, and smaller than that of oral sucker. Reproductive organs were more developed and the uterus was filled with many eggs. Eggs were oval in shape, yellowish in color, embryonated, and measured $26\text{--}30 \mu\text{m}$ in length and $16\text{--}20 \mu\text{m}$ in width.

Discussion

In the present study we found single-formed *Metagonimus* Mc which were encysted in *T.*

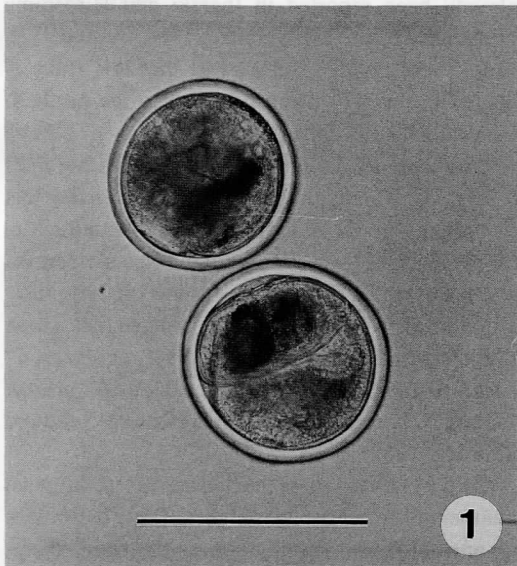


Fig. 1 Encysted metacercariae of *Metagonimus otsurui* recovered from the fins of *Tridentiger brevispinis* (Bar = $200 \mu\text{m}$).

Table 3 Location and number of adult *Metagonimus otsurui* in 8 experimentally infected golden hamsters on day 15 after administration

Total No. of		No. (%) of flukes recovered from the various regions of the small intestine			Total
animals	Mc infected*	upper	middle	lower	
8	1,600	2 (0.2)	314 (31.2)	691 (68.6)	1,007 (62.9)

*Two hundred Mc per animal were administered orally.

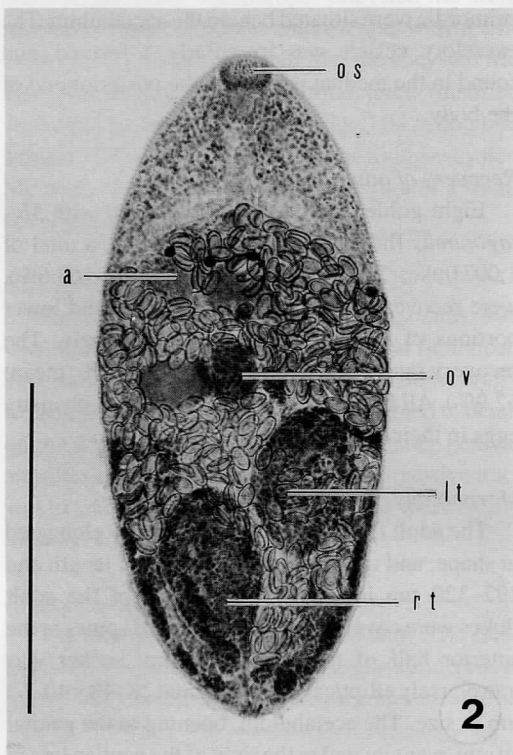


Fig. 2 Adult fluke of *Metagonimus otsurui* obtained from the small intestine of golden hamster on 15th day after infection (Bar = 400 μ m).

Ventral view, a: acetabulum, lt: left testis, os: oral sucker, ov: ovary, rt: right testis.

brevispinis collected at Lake Ogawara in Aomori Prefecture. The Mc were identified as those of *M. otsurui* by morphological features and measurements of the Mc, and adult flukes and eggs which obtained from infected golden hamsters. The

encysted Mc were spherical in shape and the thick cyst wall measured 8–18 μ m in thickness. The acetabulum was smaller than that of the oral sucker of the adult flukes. These morphological characteristics were essentially the same as those previously reported. Saito and Shimizu (1968) reported that the thickness of the cyst wall of Mc, the size of the oral sucker and acetabulum of the adult flukes were very important for specific identification of *M. otsurui*.

Until now, the Mc of *M. otsurui* have not been isolated from *T. brevispinis*. Thus, the present report is the first record that *M. otsurui* Mc are found in this fish species. In addition, this survey was conducted to confirm the geographical distribution of *M. otsurui* in Aomori Prefecture, Japan. *M. otsurui* Mc have already been reported in Ibaraki and Hiroshima prefectures (Saito and Shimizu, 1968; Saito, 1972). From these results, we suggest that this fluke is distributed widely from the south to the northern Honshu island in Japan.

The infection rate and intensity of the Mc in *T. brevispinis* were high level. It would seem that this fish species might be highly susceptible to the Mc. Many of the Mc were found in the fins, epidermis, and scales of the fish. Blackish spots on the epidermis were also observed in some infected fish. These findings are similar to those where *T. obscurus*, *C. castanea* and *C. urotaenia* infected with *M. otsurui* Mc were reported from Ibaraki Prefecture (Saito and Shimizu, 1968).

As the body length of the fish increased, the mean number of Mc per fish was higher. These data might be attributed to accumulation as the result of repeated infection. On the other hand, the infection rate of Mc showed a decrease gradually in proportion with the increase in body length. Consequently, the results might be related to the differences of the

habitats and ecological characteristics in the developmental stages of the fish. We considered that the prevalence and intensity of Mc were influenced by fish-environmental factors such as the distribution and density of cercariae and the first intermediate host of *M. otsurui* which might be easily changeable at places around Lake Ogawara.

So far as the life cycle of the genus *Metagonimus* is concerned, some species of snails could serve as the first intermediate host, and various species of freshwater fish could serve as the second intermediate hosts (Ito, 1964). To date, four species of Gobiidae freshwater fish, including *T. brevispinis* reported here, have been confirmed as the natural second intermediate host of *M. otsurui* in Japan. Concerning the final host in nature, only two Japanese water shrews have been reported in Kagoshima Prefecture by Saito *et al.* (1982). The life history of this fluke is not yet clear. Further studies are necessary to clarify the first intermediate host, the final host, and the life cycle of *M. otsurui*.

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