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A new trematode, *Metagonimus otsurui* sp. nov. from the fresh-water fishes (Trematoda: Heterophyidae)

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The genus *Metagonimus* has hitherto been known generally to be four species, *Metagonimus yokogawai* (Katsurada, 1912) Katsurada, 1912, *Metagonimus yokogawai* var. *takahashii* Suzuki, 1930, *Metagonimus minutus* Katsuta, 1932 and *Metagonimus katsuradai* Izumi, 1935.

The authors discovered a new metacercaria belonging to *Metagonimus* from the fresh-water fishes collected in Kasumigaura-lake near Miho-village, Inashiki-gun, Ibaragi prefecture, Japan. After experimental feedings of them to mammals and birds, adult flukes were obtained from the small intestines of golden hamsters. Careful observations of the fluke showed them to represent a new species and the authors proposed a new name, *Metagonimus otsurui* sp. nov. which was designed to dedicate to Professor Masamitsu Otsuru, to whom the author is much indebted for his kind continuous guidance and advice.

Materials and Methods

Metacercariae belonging to the genus *Metagonimus* were abundantly found in scales, fins and epidermis of three species of fresh-water fishes, *Tridentiger obscurus*, *Chaenogobius castanea* and *Chaenogobius urotaenia*, all of which were collected in Kasumigaura-lake near Miho-villages, Inashiki-gun, Ibaragi prefecture, 1968. These metacercariae were isolated from the tissues with the aid of dissection needles under the dissection microscope directly, or adding the artifical gastric juice to them and then incubating them under the temperature 37~38°C for 1~2 hours. Some of the metacercariae obtained were investigated morphologically and others were experimentally fed to a dog, two golden hamsters, two white leghorns and to the senior author himself. The fecal examination of experimental animals using A. M. S. III centrifugation method were carried out every day after feeding. The hamsters were sacrificed at the 5th and 8th day after feeding and searched for adult flukes in the intestine under the dissection microscope. The adult fluke obtained was fixed in Bouin's solution under the slight pressure of cover glass, stained with alum-carmin and mounted in the diluted balsam.

The encysted metacercariae isolated from the tissue of fresh-water fishes were put into the artifical intestinal juice and incubated under the temperature 37~38°C for 20~40 minutes. After this procedure, the metacercariae were washed with 0.5% NaCl solution, slightly pressed under the cover glass, and examined in living specimens.

Thirty eggs which were collected from the feces of experimental animals with A. M. S. III centrifugation method and seen in the uteri of adult flukes, respectively,

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were measured by ocular micrometer using oil immersion apparatus.

Result

Adult flukes used in the present description were obtained from the experimentally infected golden hamster on the 8th day after feeding. The flukes were fully matured, containing many eggs (94~203, aver. 148 in numbers) in uteri.

Holotype (Fig. 1 and 2): Flattened, oval tapering in anterior end of body and covered with fairly conspicuous scale-form spines in anterior half of body, measuring 0.500 mm in length and 0.269 mm in width. Oral sucker subterminal on ventral side, transversely elliptical, measuring 0.054 by 0.035 mm. Acetabulum deflecting to the right of mid-line with its long axis directed transversely, and reaching almost one third of body length from anterior end of body, openingin to genital atrium. It was measured 0.046 by 0.038 mm, smaller than oral sucker. The digestive system is consisted of a short prepharynx followed by a small, well developed pharynx and a esophagus (0.065 mm) amounting to about 2 fold as long as length of pharynx, which reaches almost two thirds of the distance from orl sucker to ace tabulum until it bifurcates into intestines. Intestines reach almost up to the posterior end of the body over the testes and terminate in two blind ceca. Pharynx is elliptical in shape, measuring 0.037 by 0.031mm. Excretory vesicle, irregularly Y formed, is found medianly occupying the posterior end of body and contain small excretory granules in it. Testes lying obliquely with each other on both lateral sides of excretory vesicle, elliptical, and fairly large, measuring 0.104 by 0.081 mm in the left and 0.119 by 0.085 mm in the right. Seminal vesicle is medianly situated at the left side of acetabulum, irregular elliptical, measuring 0.069 by 0.035 mm. Ovary is also found medianly at the level of anterior end of excretory vesicle, elliptical, measuring 0.058 by 0.042 mm. Seminal receptacle is found a little behind the right side of ovary, being situated between acetabulum and right testis, elliptical, measuring 0.062 by 0.046 mm. Uterus occupying nearly all available space of hind body from genital sucker to testes.

Paratype (Fig. 3): Forty-nine adult flukes also flattened and oval tapering in anterior end of body, of which 10 fully matured worms are 0.446~1.308 (aver. 0.479) mm in length and 0.238~0.307 (aver. 0.261) mm in width. Oral sucker 0.050~0.062 (aver. 0.054) by 0.029~0.042 (aver. 0.037) mm. Acetabulum 0.042~0.054 (aver. 0.049) by 0.035~0.040 (aver. 0.038) mm smaller than oral sucker in size, deflecting to the right of mid-line with its long axis directed transversely or diagonally. In transverse cases, the genital sucker being at the inner side of acetabulum, but in diagonal ones at the lower part of acetabulum. Pharynx elliptical or pyriform in shape, measuring 0.031~0.038 (aver. 0.035) by 0.029~0.042 (aver. 0.034) mm. Testes lying obliquely with each other on both lateral sides of excretory vesicle, elliptical, measuring 0.073 ~0.119 (aver. 0.095) by 0.058~0.085 (aver. 0.071) mm in the left and 0.088~0.123 (aver. 0.105) by 0.054~0.082 (aver. 0.075) mm in the right. Seminal vesicle varying in size, 0.029~0.067 (aver. 0.056) by 0.023 ~0.038 (aver. 0.029) mm. Ovary round or elliptical, 0.042~0.062 (aver. 0.053) by 0.031 ~0.058 (aver. 0.041) mm in size. Seminal receptacle is found always in the right side of ovary or a little behind of it. Uterus occupying nearly all available space of hind body from genital sucker to testes, sometimes extending to upper part of acetabulum.

Fecal examination of experimental animals were carried out every day after feeding, and the eggs were found in fecal specimen of golden hamster after the 8th day, but not from a man, a dog and two white leghorns for 14 days. Very small eggs (Fig. 6~8) oval in shape, pale yellow

or pale yellowish brown in color. At the upper pole there is a small operculum, and at the lower pole sometimes a nodule. The development of larvae begins even in uterus. When laid, they already contain a fully-formed miracidium. The size of eggs in uterus was 0.028~0.033 (aver. 0.030) mm in length, 0.015~0.018 (aver. 0.017) mm in width, and in the golden hamster feces 0.028~0.033 (aver. 0.031) mm in length, 0.016~0.018 (aver. 0.017) mm in width.

The metacercariae are found encysted in scales, fins and epidermis of *Tridentiger obscurus*, *Chaenogobius castanea* and *Chaenogobius urotaenia*. They are frequently visible as the blackish spots to the naked eye. The cyst wall is spherical in shape, measuring 0.169~0.204 mm in diameter, with transparent layer; its thickness is very thick, measuring 0.007~0.013 mm.

The metacercariae (Fig. 9~11) taken out of their cysts are flat, oval or elliptical in shape, measuring 0.400~0.431 (aver. 0.419) mm by 0.200~0.215 (aver. 0.204)mm. The surface of the body is covered with minute scale-form spine nearly toward posterior end of the body. A few pigmented corpuscles are found in the body. Oral suker is elliptical, measuring 0.044~0.052 (aver. 0.049) by 0.035~0.046 (aver. 0.039) mm, located ventrally in anterior subterminal end. Acetabulum is elliptical, measuring 0.037~0.042 (aver. 0.039) by 0.031~0.035 (aver. 0.033) mm, deflected to the right of mid-line. The digestive system is consisted of a short pre-pharynx followed by a pharynx and a relatively long esophagus, which reaches almost two thirds of the distance from oral sucker to acetabulum until it bifurcates into intestines. Pharynx is rather pyriform in shape, measuring 0.031~0.035 (aver. 0.032) mm by 0.023~0.031 (aver. 0.027) mm. Intestines reach almost behind the testicular primordia and terminate in two blind ceca. Excretory vesicle, irregularly Y formed, is found medianly occupying the posterior end of the body, being constricted anterolaterally owing to presence of testicular primordia. Excretory granules in it are variable in size, the largest being 0.004~0.006 mm in diameter. Testicular primordia laying obliquely with each other on both lateral sides of excretory vesicle, elliptical, fairly large, measuring 0.038~0.046 (aver. 0.041) by 0.035~0.038 (aver. 0.036) mm in the left, 0.042~0.050 (aver. 0.044) by 0.035~0.038 (aver. 0.036) mm in the right. Ovaria n primordia irregularly elliptical, measuring 0.025~0.031 (aver. 0.027) by 0.023~0.027 (aver. 0.025) mm, laying sinistrolaterally between acetabulum and excretory vesicle.

Final hosts: golden hamster (experimentally)

Location: small intestine

Second intermediate hosts: *Tridentiger obscurus* (Temminck et Schlegel), *Chaenogobius castanea* (O'Shaughessy), *Chaenogobius urotaenia*(Hilgendorf).

Type specimens: They are deposited in the Department of Medical Zoology, Niigata University School of Medicine, Niigata, Japan.

Locality: Kasumigaura-lake near Miho-village, Inashiki-gun, Ibaragi Prefecture, Japan.

Discussion

The acetabulum of this new species is smaller than the oral sucker, differing from three known species of *M. yokogawai*, *M. yokogawai* var. *takahashii* and *M. minutus*. Accordingly, it is most necessary to compare the new species with *M. katsuradai* which show close resemblance in the size of acetabulum. First of all, the new species differs from *M. katsuradai* in the position of seminal receptacle, that is, the former locate at the right side of ovary between acetabulum and right testis, but the latter at the left side of ovary between seminal vesicle and left testis (Izumi, 1935; Kurokawa, 1939). The seminal receptacle of the remaining 39 specimens also was located at the same situation, as was seen in the holotype and 10 paratypes. According to

Izumi (1935), the testes of *M. katsuradai* were measured 0.058 by 0.054 mm in the left and 0.060 by 0.056 mm in the right both in average size; Kurokawa (1939) repored nearly the same size in rats as Izumi did. But testes of the new species were measured 0.095 by 0.071 mm in the left and 0.105 by 0.075 mm in the right both in average size, showing much larger than *M. katsuradai* in size, and the figures (average of 10 specimens, 0.070 by 0.055 mm in the left, 0.080 by 0.064 mm in the right) of the new species (Fig. 4) fixed in Bouin's solution without pressure were also larger than data of Izumi (1935) and Kurokawa (1939).

In the metacercaria there is a clear-cut difference between this species and four known species. The cyst wall of new species (0.007~0.013 mm) is much thicker than that of the latter species(0.002~0.003 mm), the testicular primordia of the former being larger than those of the other species. Second intermediate host of *M. katsuradai* were reported to be encysted in *Acheilognathus lanceolata*, *Pseudorasbora parva* and *Zacco platypus* by Izumi (1935). Okabe (1940) reported that *M. katsuradai* were found in *Pseudoperilampus typus*, *Acheilognathus lanceolata*, *Acheilognathus rhombea* and *Tribolodon hakonensis*, but not in *Tridentiger obscurus* and *Chaenogobius urotaenia* collected in the same river. On the other hand, this species was found in *Tridentiger obscurus*, *Chaenogobius castanea* and *Chaenogobius urotaenia*, but not in 50 specimens of *Achielognathus lanceolata* collected in the same lake.

The eggs were not distinguished from *M. yokogawai* and *M. katsuradai*, but could be from *M. minutus* (0.023 by 0.013 mm by Katsuta, 1932) in size.

Summary

A metacercaria belonging to the genus *Metagonimus* was discovered from fresh-water fishes, *Tridentiger obscurus*, *Chaenogobius castanea* and *Chaenogobius urotaenia*, which were collected in Kasumigaura-lake near Miho-village, Inashiki-gun, Ibaragi prefecture, Japan, 1968. Some of the metacercariae obtained were investigated morphologically and the others were experimentally fed to a dog, two golden hamsters and two white leghorns, to the senior author himself, and adult flukes were obtained from the small intestines of golden hamsters. After morphological studies and detailed comparisons with four known species of the genus *Metagonimus*, the authers proposed the name *Metagonimus otsurui* sp. nov. for this new fluke.

Acknowledgement

The authors wish to express his sincere appreciation to Prof. M. Otsuru, Chief of the Department of Medical Zoology, Niigata University, School of Medicine, for guidance throughout the course of this investigation, and to Dr. S. Miyamoto Director of Miyamoto hospital, Tsukuba-town, Ibaragi prefecture, for his cooperation to the collection of fresh-water fishes.

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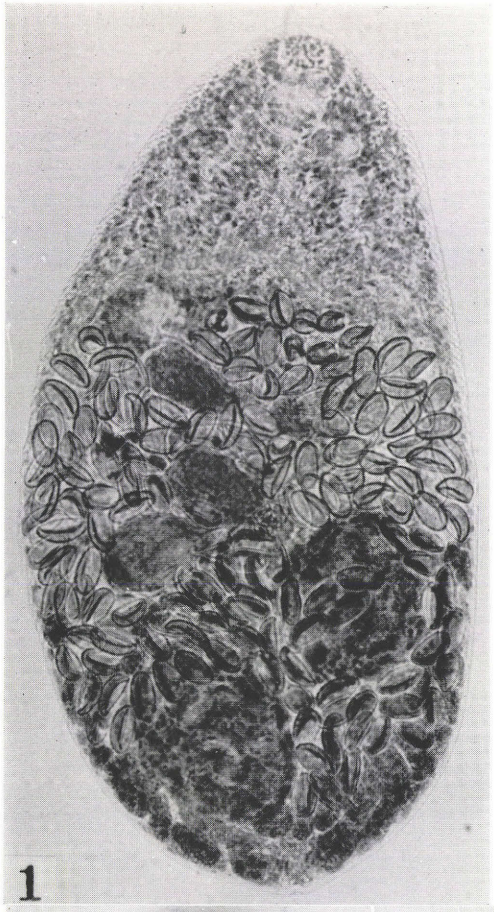

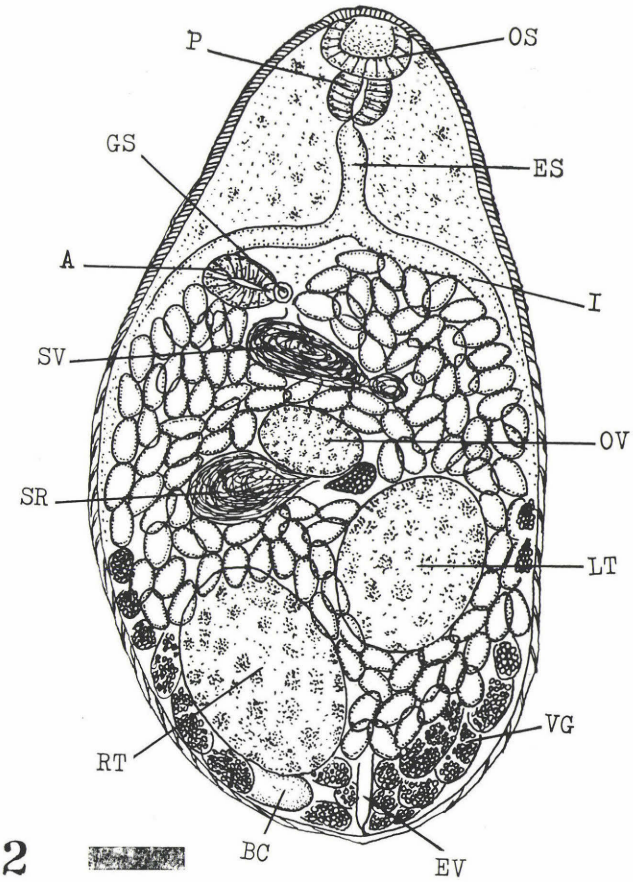
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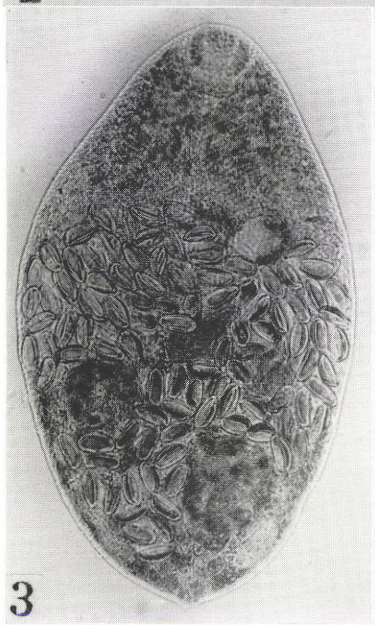

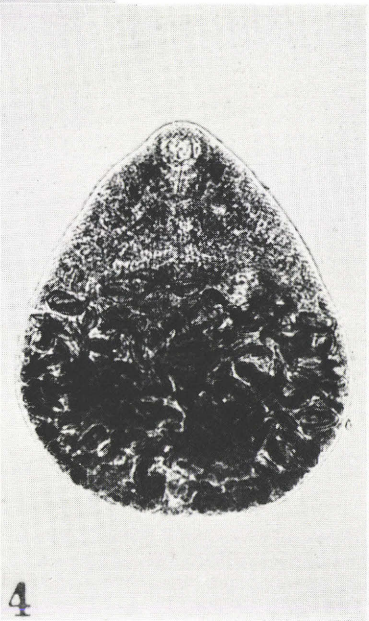

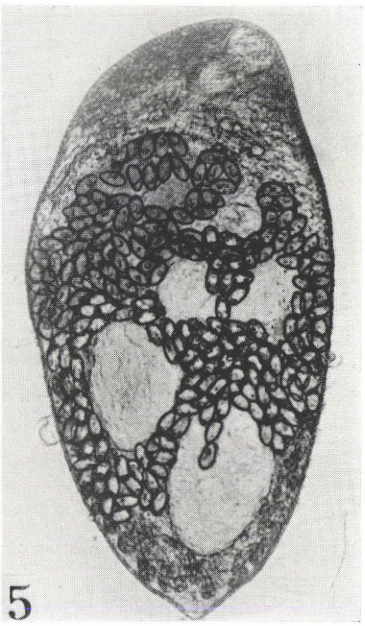

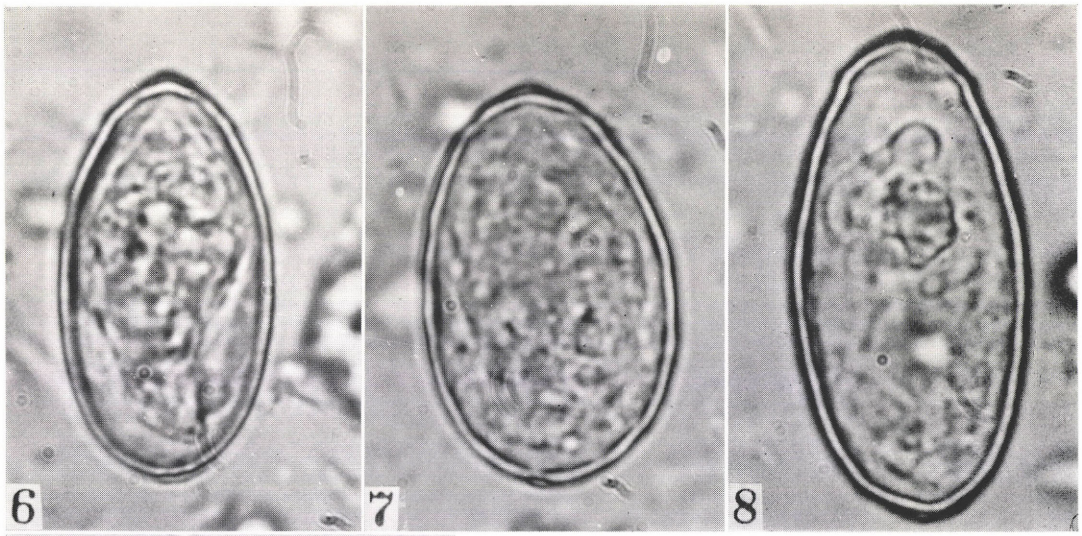
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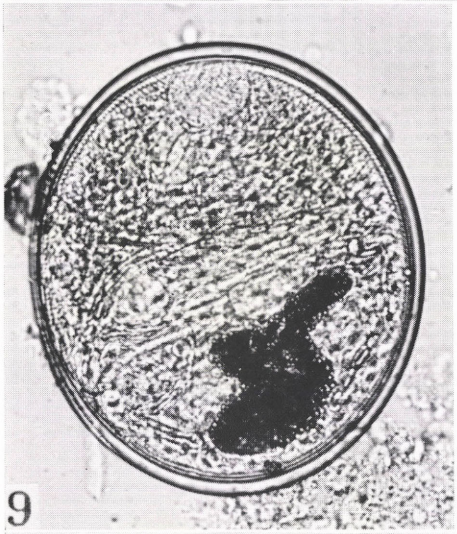
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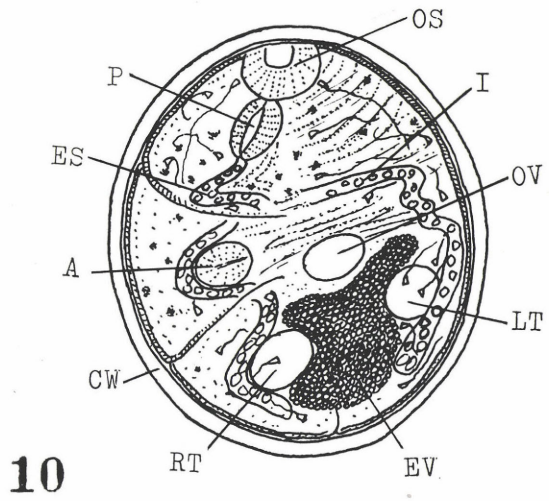
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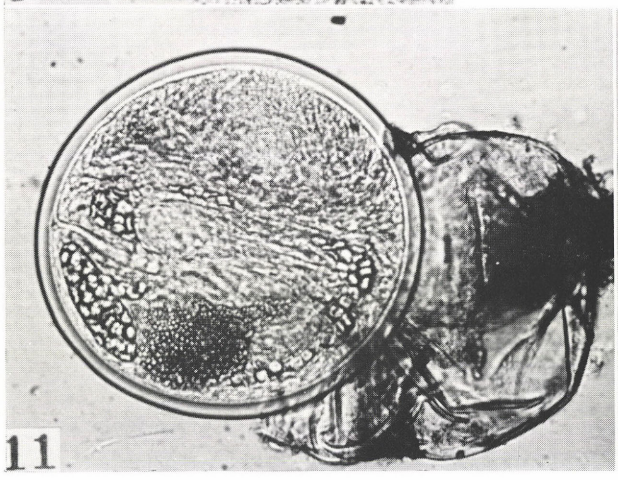
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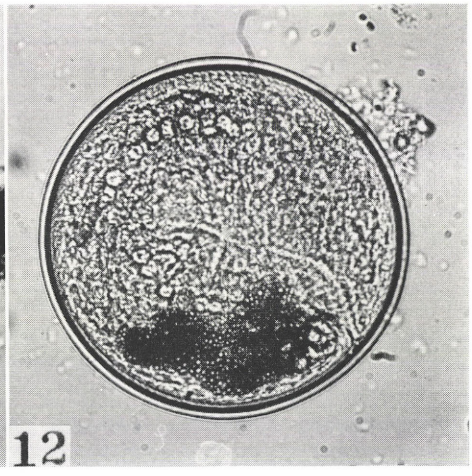
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Explanation of Plates

Fig. 1 Ventral view of holotype of *Metagonimus otsurui* sp. nov.

Fig. 2 Systematic drawing of the holotype.
A: Acetabulum, BC: Blind ceca, ES: Esophagus, EV: Excretory vesicle, I: Intestine, GP: Genital sucker, LT: Left testis, OS: Oral sucker, OV: Ovary, P: Pharynx, RT: Right testis, SR: Seminal receptacle, SV: Seminal vesicle, VG: Vitelline glands

Fig. 3 Dorsal view of paratype of *Metagonimus otsurui* sp. nov.

Fig. 4 Adult fluke of *Metagonimus otsurui* sp. nov., which was fixed in Bouin's solution without pressure and stained with alum-carmin.

Fig. 5 The living adult fluke under the slight pressure of cover glass.

Figs. 6, 7 & 8 The eggs collected from feces of the experimental animals by A. M. S. III centrifugation method.

Figs. 9 & 12 Metacercariae of *Metagonimus otsurui* sp. nov.

Fig. 10 Systematic drawing of the metacercaria.
A: Acetabulum, ES: Esophagus, EV: Excretory vesicle, I: Intestine, LT: Left testicular primordia, OS: Oral sucker, OV: Ovarian primordia, P: Pharynx, RT: Right testicular primordia

Fig. 11 The metacercaria with layer of host origin.

淡水魚に寄生する *Metagonimus* 属吸虫の新種 *Metagonimus otsurui* sp. nov. について

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Metagonimus 属吸虫の種名にはいろいろの説があるが，現在では一応 *Metagonimus yokogawai* (Katsurada, 1912) Katsurada, 1912, *Metagonimus yokogawai* var. *takahashii* Suzuki, 1930, *Metagonimus minutus* Katsuta, 1932 および *Metagonimus katsuradai* Izumi, 1935 の4種が知られている。

著者は1968年1月，茨城県霞ケ浦のチチブ，ビリンゴおよびウキゴリに寄生していたメタセルカリアとその動物感染実験（ハムスター）からえた成虫および虫卵の形態を詳細に観察した結果，下記のように *Metagonimus* 属吸虫の既知種のどれとも異なっていたので新種と判断し，その学名を *Metagonimus otsurui* sp. nov., 和名をオオツルキュウチュウと提称した。

1. 本成虫は腹吸盤が口吸盤より小さく，この点では *M. yokogawai*, *M. yokogawai* var. *takahashii* および *M. minutus* と異なり，*M. katsuradai* に似ている。

2. 本成虫の受精嚢は腹吸盤と右睾丸の間，卵巣の右側に存在しているが，*M. katsuradai* のそれは貯精嚢と左睾丸の間，卵巣の左側にある。

3. 本成虫の睾丸は左（平均 0.095×0.071 mm）右（平均0.105×0.075mm）ともに *M. katsuradai* よりもはるかに大きい。

4. 本メタセルカリアの被包壁の厚さは0.007～0.013 mm で，既知種の 3～4 倍もある。

5. 本メタセルカリアの睾丸原基は左（平均 0.041×0.036 mm）右（平均 0.044×0.036 mm）ともに既知種のいずれよりも大きい。

6. 本吸虫の第二中間宿主はチチブ，ビリンゴおよびウキゴリの3種が知られ，その他の淡水魚からまだ検出されていない。

7. 本虫卵の大きさ平均（0.030～0.031×0.017 mm）は *M. yokogawai* および *M. katsuradai* と近似しているが，*M. minutus* とは異なるようである。