

## Morphology and Life Cycle of *Metagonimus otsurui* (Digenea, Heterophyidae) from Nara, Japan

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**Abstract** The life cycle of *Metagonimus otsurui* Saito & Shimizu, 1968 (Digenea, Heterophyidae) was studied in the Takami River at Kotsukawa, Higashiyoshino Village, Nara Prefecture, Japan. Metacercariae were found encysted in *Rhinogobius flumineus* (Mizuno) (Osteichthyes, Gobiidae). Adults were found in the small intestine of *Chimarrogale himalayaica* (Gray) (Mammalia, Insectivora, Soricidae). Metacercariae from naturally infected gobies were experimentally fed to golden hamsters, from the small intestine of which ovigerous adults were recovered 8 days after infection. Cercariae in daughter rediae were obtained from *Semisulcospira libertina* (Gould) and *S. reiniana* (Brot) (Gastropoda, Pleuroceridae). They were experimentally exposed to *R. flumineus* and fully formed metacercariae were recovered 24 and 38 days after infection. Although the prevalence and abundance of metacercariae were low in this experiment, it is considered that the cercaria is that of *M. otsurui*. The adults, metacercariae, daughter redia and cercaria are described. The life cycle of the species is briefly discussed.

**Key words:** *Metagonimus otsurui*, Digenea, Heterophyidae, morphology, life cycle, Japan.

### Introduction

Members of the genus *Metagonimus* Katsurada, 1912 (Digenea, Heterophyidae) are parasitic in the small intestine of mammals and birds in eastern and southeastern parts of Asia and in southeastern parts of Europe (see Shimazu, 1999). Saito & Shimizu (1968) found metacercariae of a species of the genus in *Chaenogobius castaneus* (O'Shaughnessy) (now in *Gymnogobius*), *C. urotaenia* (Hilgendorf) (now in *Gymnogobius*) and *Tridentiger obscurus* (Temminck & Schlegel) (Osteichthyes, Gobiidae) all from Kasumigaura, a brackish-water lake in Ibaraki Prefecture, Japan. They experimentally fed the metacercariae to golden hamsters and recovered adults from the small intestine of the golden hamsters. They described a new species, *M. otsurui*, on the basis of the adults. The fish dealt with

as *T. obscurus* by them may have been *T. brevispinis* Katsuyama, Arai & Nakamura (see Prince Akihito, 1987). Oyamada *et al.* (1996) and Shimazu (1999) obtained metacercariae of *M. otsurui* from *G. castaneus* and *T. brevispinis* from Lake Ogawara, a brackish-water lake in Aomori Prefecture, Japan. Saito *et al.* (1982) recorded adults of *M. otsurui* from *Chimarrogale himalayaica* (Gray) (Mammalia, Insectivora, Soricidae) from the Kawauchi River and its tributary [or tributaries?] in Tsuruda, Kagoshima Prefecture, Japan. However, they have not yet described their adults. This paper reports adults, metacercariae, daughter rediae and cercariae of *M. otsurui* all found in a mountain river in Nara Prefecture, Japan.

This study was carried out in part at the Higashiyoshino Laboratory of Nara Women's University, Higashiyoshino Village, Nara Prefecture.

## Materials and Methods

### Natural second intermediate host

Gobies of *Rhinogobius flumineus* were collected in the Takami River at Kotsukawa, Higashiyoshino Village, Nara Prefecture, Japan, several times at irregular intervals from July 1999 to August 2000. Metacercariae were dug up from the fish with a pair of fine tweezers.

### Natural final host

Two individuals of *Chimarrogale himalayica* were obtained dead by a fishing trap at the same locality. The small intestine of one obtained on 17 March 1998 was examined fresh for flukes. That of the other obtained on 19 July 1999 was examined after having been preserved in 10% formalin.

### Experimental infection of metacercariae to golden hamsters

Metacercariae from naturally infected gobies were orally given by a stomach tube to two golden hamsters. The golden hamsters were examined for flukes 8 days after infection.

### Natural first intermediate hosts

Snails of *Semisulcospira libertina* (Gould) and *S. reiniana* (Brot) (Gastropoda, Pleuroceridae) were collected at the same locality several times at irregular intervals from July 1999 to August 2000. They were first individually tested for emergence of cercariae for a few days and finally crashed for parthenitae and cercariae.

### Experimental infection of cercariae to *Rhinogobius flumineus*

Apparently fully formed cercariae from crashed snails (3 snails of *S. libertina* and 4 snails of *S. reiniana*) were exposed to 45 gobies (30–48 mm in standard body length) of *R. flumineus*. These gobies were collected at the same locality and they proved to be free in the fins from any metacercariae from natural infection prior to the experiment. Metacercariae obtained from the fins of the experimental gobies 38 days

after infection were orally given to one golden hamster. The golden hamster was examined 8 days after infection.

### Specimens and methods

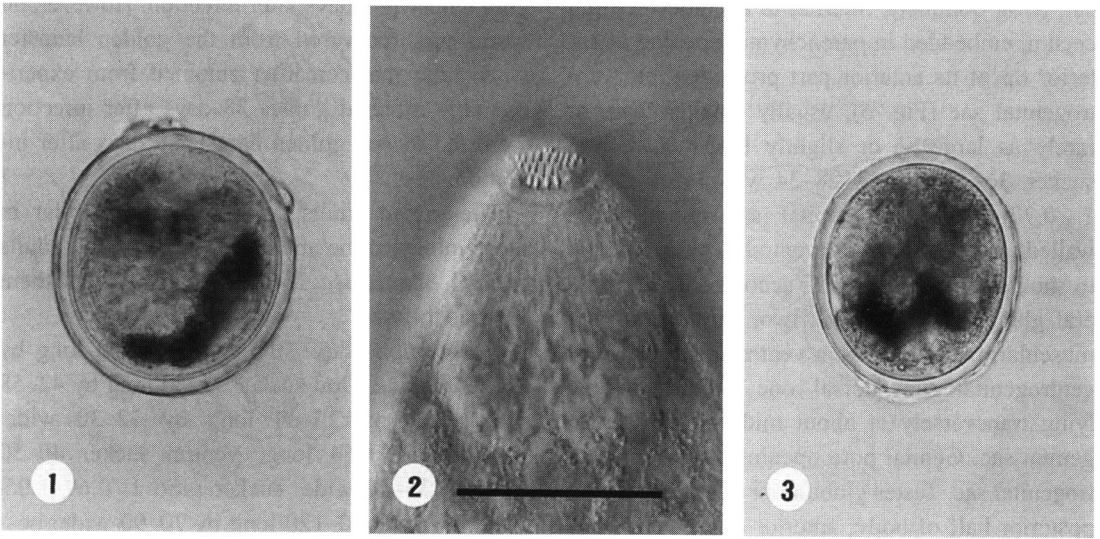
Flukes found in *C. himalayica* examined fresh were slightly flattened, fixed in AFA, stained with Heidenhain's iron hematoxylin. Those found in the other *C. himalayica* were stained with Mayer's hematoxylin. Those found in the experimental golden hamsters were slightly flattened, fixed in 70% ethanol and stained with Grenacher's alum carmine. Parthenitae and cercariae obtained from crashed snails were fixed in hot 10% neutralized formalin and stained with Mayer's hematoxylin. Some were slightly flattened, fixed in AFA and stained with Heidenhain's iron hematoxylin. Metacercariae were pressed out of their cysts, slightly flattened, fixed in 70% ethanol and stained with Grenacher's alum carmine. The stained specimens were mounted in Canada balsam. Some parthenitae, cercariae and metacercariae were observed alive.

Drawings were made with the aid of a drawing tube, some with supplements with free-hand details from living material. Measurements are given in micrometers unless otherwise stated. The sucker ratio means the ratio of the width of the oral sucker to the length (or larger diameter) of the ventral sucker (see Shimazu, 1999). Representatives of the specimens studied have been deposited in the National Science Museum, Tokyo (NSMT-PI 4616–4621). The type and voucher specimens of *M. otsurui* were also reexamined (see Shimazu, 1999).

## Results

### Metacercariae from naturally infected *Rhinogobius flumineus*

A heavy black spot was usually noted around a metacercarial cyst in the skin of the body and fins and scales of *R. flumineus*. Out of 26 gobies collected on 12 and 13 August 2000, 20 harbored 1 to 27 (mean, 4.9) black spots per fish, but the remaining 6 were free from any black spots. The



Figs. 1–3. *Metagonimus otsurui*. 1. Metacercaria from *Rhinogobius flumineus* from the Takami River at Kotsukawa, Higashiyoshino Village, Nara Prefecture, on 9 October 1999. Natural infection, living, cyst size 198 by 180  $\mu\text{m}$ , cyst wall 12  $\mu\text{m}$  thick. 2. Cercaria from *Semisulcospira libertina* from the same locality on 12 August 2000. Natural infection, living, anterior part of body, showing oral spines, ventral view. Scale bar=50  $\mu\text{m}$ . 3. Metacercaria recovered from the pectoral fin of *R. flumineus*. Experimental infection, 24 days after infection, living, cyst size 174 by 150  $\mu\text{m}$ , cyst wall 10  $\mu\text{m}$  thick.

morphology of whole-mounted metacercariae was as follows, with measurements of five specimens.

**Description** (Figs. 1 and 4). Cyst a little ellipsoidal and flattened, 160–198 by 150–180; cyst wall thick, 7–13 thick.

Body ovate, spinose, 190–310 long by 110–176 wide; brown pigmentation of body heavy. Oral sucker subventral, 30–44 long by 36–44 wide. Prepharynx almost absent. Pharynx 20–30 long by 18–30 wide. Esophagus 28–40 long, bifurcating slightly anterior to midlevel of body. Ceca including many pale disks about 8 in diameter by 2–3 thick, extending posteriorly beyond testes to near posterior end of body. Ventral sucker internal to shoulder of right cecum, 26–36 long by 22–30 wide; sucker ratio 1: 0.71–0.86. Ovary median, slightly posterior to midlevel of body, 14–22 long by 16–20 wide. Testes slightly diagonal; anterior or left testis 26–44 long by 22–36 wide; posterior or right one 30–54 long by 28–46 wide. Excretory vesicle Y-shaped, containing fine granules; flame-cell formula  $2[(2+2+2)+$

$(2+2+2)]=24$ ; main collecting canals connected subterminally to respective arms of excretory vesicle, ciliated in posteriormost parts.

#### Adults from *Chimarrogale himalayica*

A total of 33 immature and 23 (plus 3 broken?) gravid flukes were found in the small intestine of two specimens of *C. himalayica*. All these flukes were morphologically similar and regarded as belonging to a single species. The morphology of them was as follows, with measurements of 10 gravid specimens.

**Description** (Figs. 5 and 6). Body oval, 304–440 long by 190–250 wide (Fig. 5). Tegument covered with scales. Brown body pigmentation rather heavy. Transverse nerve commissure located posterodorsal or dorsal to pharynx. Oral sucker elliptical, subterminal, 36–44 long by 48–60 wide. Prepharynx very short. Pharynx elliptical, 22–34 long by 24–32 wide. Esophagus 30–90 long, bifurcating at about junction of anterior and middle thirds of body. Ceca ventral, extending into posttesticular region. Ventral sucker ellipti-

cal, lying obliquely, internal to shoulder of right cecum, embedded in parenchyma, opening at anterior tip of its anterior part protruded into ventrogenital sac (Fig. 6), usually smaller than, or rarely as large as or slightly larger than, oral sucker, 36–46 long by 28–34 wide; sucker ratio 1: 0.77–1.10. Ventrogenital sac large, thick-walled, anterosinistral to ventral sucker, internal to shoulder of right cecum, accompanied by several gland cells. Gonotyls two; ventral one tall, muscular, projecting from ventrosinistral base of ventrogenital sac; dorsal one short, crescent, lying transversely at about midlevel of ventrogenital sac. Genital pore opening ventral to ventrogenital sac. Testes globular or oval, diagonal in posterior half of body; anterior or left testis 60–86 long by 50–82 wide; posterior or right one 68–90 long by 50–84. Seminal vesicle bipartite, postbifurcal, 54–110 long by 34–50 wide. Pars prostatica small, with prostatic cells dispersed around it. Cirrus pouch absent. Ejaculatory duct short, uniting with metraterm to form a genital atrium like a hermaphroditic duct opening into ventrogenital sac between dorsal gonotyl and ventral sucker. Ovary almost globular, median, almost equatorial, between seminal vesicle and testes, 30–54 in diameter. Ootype-complex not worked out. Seminal receptacle dorsal, posterodextral to ovary, 54–60 long by 40–50 wide. Uterus occupying all available space between intestinal bifurcation and testes, extending posteriorly between testes to embrace anterior testis; metraterm short. Eggs operculate, fully embryonated in distal part of uterus, 26–30 by 14–18 in balsam. Vitelline follicles dorsal, clavate, distributed between midlevel of anterior testis and posterior end of body. Excretory vesicle ventral, Y-shaped, bifurcating posterior to ovary, with short arms; excretory pore posteroterminal.

#### **Adults from experimentally infected golden hamsters**

When the metacercariae from the naturally infected gobies were fed to two golden hamsters, a total of 19 gravid adults were recovered from the posterior third part of the small intestine of the

gold hamsters 8 days after infection. However, no worm was recovered from the golden hamster when nine metacercariae obtained from experimentally infected gobies 38 days after infection were fed to one golden hamster 8 days after infection.

The gravid adults recovered were similar in morphology to the above-described gravid adults from *C. himalayica*. Measurements of 10 of them were as follows.

*Description* (Fig. 7). Body 346–500 long by 260–300 wide. Oral sucker 37–50 long by 42–58 wide. Pharynx 26–34 long by 22–30 wide. Esophagus 30–54 long. Ventral sucker 40–50 long by 34–46 wide; sucker ratio 1: 0.69–1.05. Anterior testis 80–120 long by 70–90 wide; posterior one 80–120 long by 80–100 wide. Seminal vesicle 80–100 long by 30–40 wide. Ovary 44–60 long by 56–80 wide. Seminal receptacle 66–140 long by 50–100 wide. Eggs 26–30 by 16–20 in balsam.

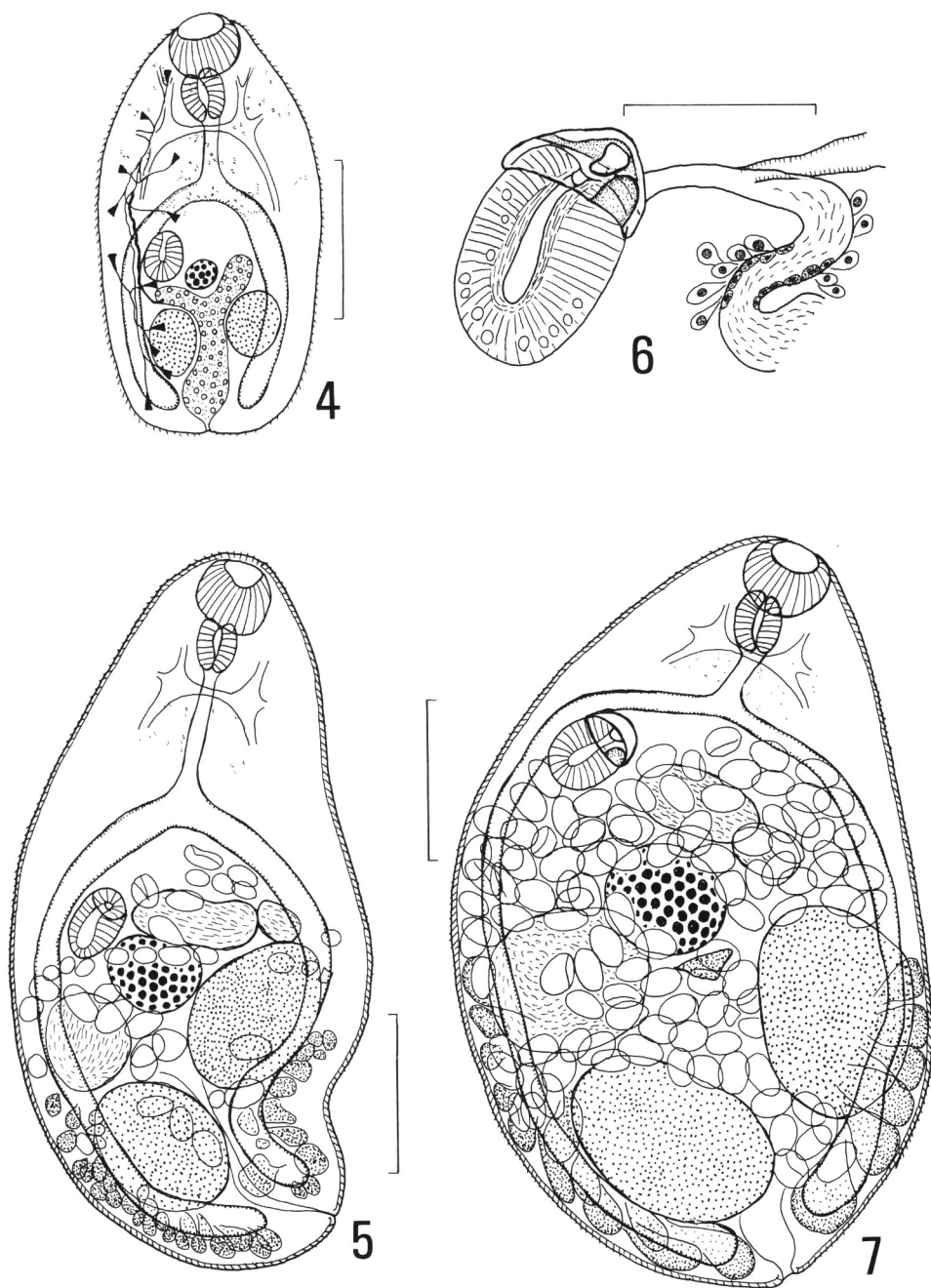
#### **Metacercariae from experimentally infected *Rhinogobius flumineus***

A single metacercaria was found in the pectoral fin of one goby 24 days after infection. The cyst measured 174 by 150, and the cyst wall was 10 thick (Fig. 3).

A total of nine metacercariae were obtained from the fins of 39 remaining gobies. The cysts were 160–192 by 136–159, and the cyst walls were about 10 thick. The ceca include disks about 8 by 2–3. No black spot was seen around the metacercarial cyst. As already mentioned above, their experimental infection to one golden hamster failed.

#### **Daughter rediae and cercariae from naturally infected snails**

The prevalence of the cercaria was not so high. From 13 to 17 August 2000, for example, 3 of 167 snails (12–50 mm in shell height) of *S. libertina* and 4 of 1181 snails (10–48 mm in shell height) of *S. reiniana* from the same locality were infected with a pleurolophocercous cercaria, respectively. During this study, no sporo-



Figs. 4–7. *Metagonimus otsurui*. 4. Excysted metacercaria from *Rhinogobius flumineus* from the Takami River at Kotsukawa, Higashiyoshino Village, Nara Prefecture, on 13 August 2000. Natural infection, living, ventral view. 5. Adult from the small intestine of *Chimarrogale himalayica* from the same locality on 17 March 1998. Natural infection, whole-mounted, entire body, ventral view. 6. Adult from the small intestine of *C. himalayica* from the same locality on 17 March 1998. Natural infection, whole-mounted, terminal genitalia, ventrogenital sac and ventral sucker, ventral view. 7. Adult from the small intestine of a golden hamster. Experimental infection, 8 days after infection, whole-mounted, entire body, ventral view. Scale bars=50  $\mu$ m in Fig. 6; 100  $\mu$ m in Figs. 4, 5 and 7.

cysts nor mother rediae were obtained. Rediae were in various developing stages and larger ones contained only cercariae in various developing stages. They were regarded as daughter rediae. Well-formed large cercariae bore eyespots in their rediae. The morphology of the daughter rediae and cercariae were as follows, with measurements of 10 each of hot formalin-fixed specimens.

*Description of daughter rediae* (Fig. 8). Body elongate, 663–955 long by 117–136 wide. Several hairs seen on anterior end of body. Pharynx spherical, 26–30 long by 30–34 wide. Cecum short, extended or S-shaped, 52–66 long. Birth pore present near pharynx. Flame-cell formula  $2[(1+1)+(1+1)]=8$ .

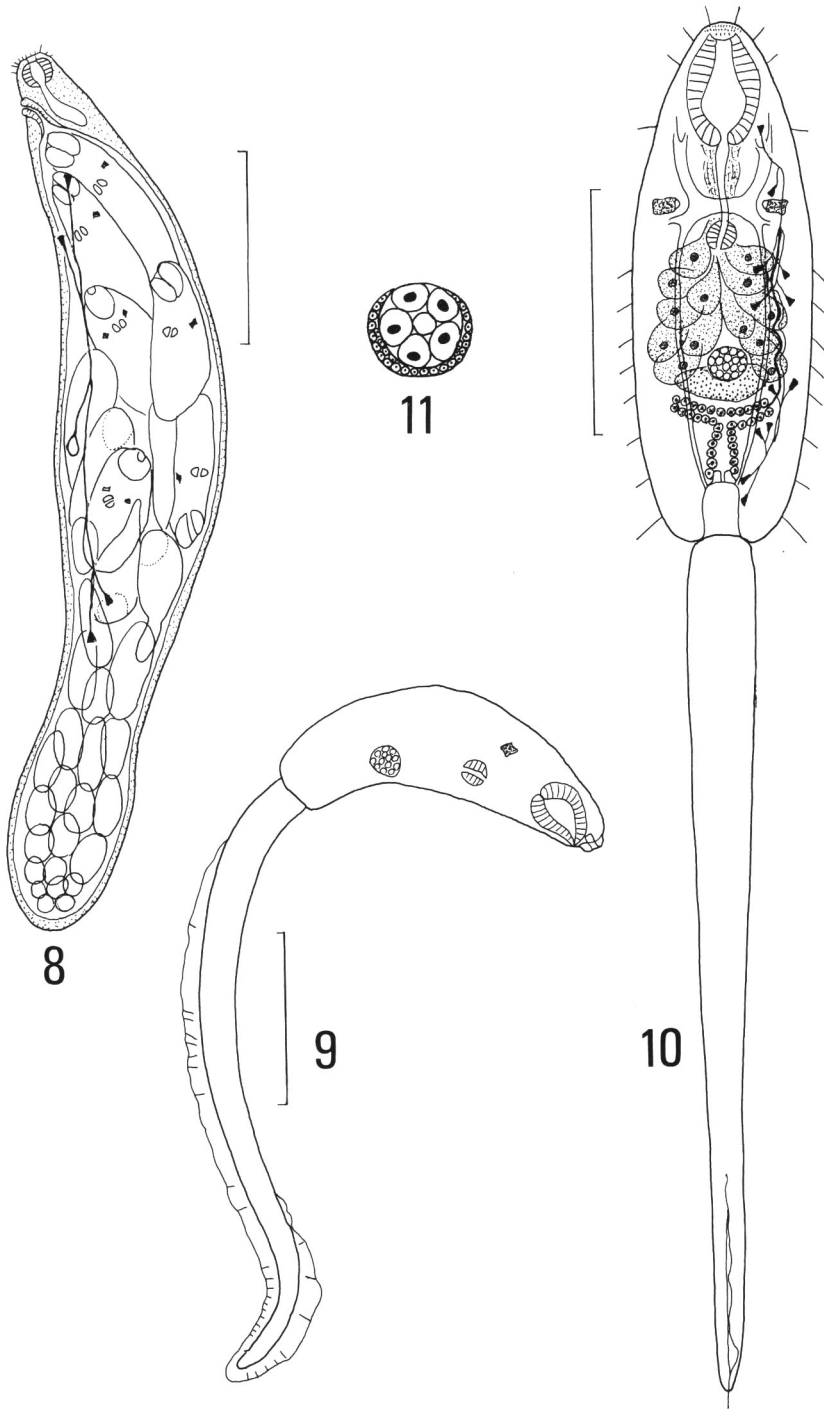
*Description of cercariae* (Figs. 2 and 9–11). Of pleurolophocercous type. Body elongate, finely spinose, 188–210 long by 66–74 wide; at least 12 pairs of sensory hairs seen on lateral margins of body. Brown pigmentation of body heavy. Tail inserted deeply in tail socket, 350–390 long by 23–31 wide; finfold occupying about 30% and 85% of tail length from distal end on ventral and dorsal sides, respectively, 10 height. A pair of eyespots dorsal, present at about junction of anterior and middle thirds of body. Transverse nerve commissure dorsal to prepharynx; a pair of posterior trunks reaching to base of tail socket. Penetration glands consisting of anterior 3 and posterior 4 pairs on each side of body, elliptical, small, 18–30 by 10–20, all similar in texture, lying along median line between pharynx and midlevel of ventral sucker; ducts of anterior and posterior glands on each side of body running forwards in an outer and an inner bundle, respectively, first ventral to glands and then dorsal to transverse nerve commissure and oral sucker to open separately in a depression at anterior tip of body. Cystogenous glands present between pharynx and posterior end of body, numbering about 36 on each side of body. Oral sucker elliptical, subterminal, 38–44 long by 26–28 wide; oral spines numbering 4 in first or posteriormost row (Figs. 2 and 10). Prepharynx long. Pharynx subglobular, 10–12 long by 12–14 wide, posterior to trans-

verse nerve commissure. Esophagus and ceca not differentiated yet. Ventral sucker globular, median, 14–20 in diameter, at about junction of middle and posterior thirds of body, including many small cells in fully formed stage (Fig. 10) but only 5 large tall cells in younger developmental stages (Fig. 11). Genital primordium depressed orbicular, dorsal to ventral sucker, 26–40 long by 20–30 wide. Excretory vesicle usually Y-shaped, epithelial, thick-walled, with short arms; main collecting canals connected subterminal to respective arms of excretory vesicle, not yet ciliated in posteriormost parts; caudal excretory canal extending short distance into tail, bifurcating to open outside through two lateral primary pores (these hardly seen in fully formed cercariae); flame-cell formula  $2[(2+2+2)+(2+2+2)]=24$ .

## Discussion

The genus *Metagominus* are composed of eight described species at present: *M. yokogawai* (Katsurada, 1912) Katsurada, 1912; *M. romanicus* (Ciurea, 1915) Ciurea, 1924; *M. takahashii* Suzuki in Takahashii, 1929; *M. minutus* Yokogawa in Katsuta, 1932; *M. katsuradai* Izumi, 1935; *M. otsurui* Saito & Shimizu, 1968; *M. miyatai* Saito, Chai, Kim, Lee & Rim, 1997; and *M. hakubaensis* Shimazu, 1999 (see Saito *et al.*, 1997; Shimazu, 1999). Saito *et al.* (1997) question whether *M. parvus* (Ciurea, 1915) Ciurea, 1924 and *M. dobrogiensis* (Ciurea, 1915) Ciurea, 1924 are synonymous with *M. romanicus*.

The present adults from the naturally infected *C. himalayica* and those from the experimentally infected golden hamsters are very similar in morphology and measurements although the former are smaller as a whole than the latter because the latter were slightly flattened before fixation. It seems certain that they belong to a single species. Out of the eight species, the oral sucker is larger than the ventral sucker in the adults of *M. katsuradai*, *M. otsurui* and *M. hakubaensis*; but smaller in the five others. The present adults are most closely similar in morphology and measurements to *M. otsurui* as described by Saito &



Figs. 8–11. Daughter redia and cercariae of *Metagonimus otsurui* from *Semisulcospira libertina* from the Takami River at Kotsukawa, Higashiyoshino Village, Nara Prefecture, on 12 August 2000. 8. Daughter redia, fixed in hot formalin, lateral view. 9. Fully-formed cercaria, fixed in hot formalin, lateral view. 10. Fully-formed cercaria, fixed in hot formalin, ventral view. 11. Immature cercaria, fixed in hot formalin, ventral sucker and genital primordium, ventral view. Scale bars=100  $\mu$ m in Figs. 9 and 10; 200  $\mu$ m in Fig. 8.

Shimizu (1968) and to the type and voucher specimens of the species. Metacercariae of *M. otsurui* encyst in gobiids and their cyst walls are very thick (Saito & Shimizu, 1968; Oyamada *et al.*, 1996) as in the present metacercaria. The flame-cell formula is  $2[(2+2+2)+(2+2+2)]=24$  in the metacercaria of *M. otsurui* (see Shimazu, 1999). Consequently, the present adult from *C. himalayica* and metacercaria from *R. flumineus* are identified as *M. otsurui*.

When the present cercariae from *S. libertina* and *S. reiniana* were experimentally exposed to *R. flumineus*, only 10 metacercariae were recovered from the fins of the 40 gobies 24 and 38 days after infection. Gobies without any metacercariae in the fins were used for the experiment but the 10 metacercariae were found in the fins of the gobies. In the cyst size and thickness of the cyst wall, they were almost the same as those from natural infection in *R. flumineus* and those as described by Saito & Shimizu (1968) and Oyamada *et al.* (1996). Therefore, it is considered that the 10 metacercariae were from the experimental infection, which means that the present cercaria is that of *M. otsurui*. By the way, 29 metacercariae including ones from natural infection were obtained from the parts other than the fins from 38 gobies 38 days after infection. It is unknown why the prevalence and abundance of metacercariae in the fins of *R. flumineus* were very low in the present experiment.

In the present study, besides the present cercaria, two other pleurolophocercous cercariae were obtained from *S. libertina* and *S. reiniana*. One had four oral spines, small subglobular penetration glands and the flame-cell formula of  $2[(3+3+3)+(3+3+3)]=36$  (NSMT-PI 4622–4623). This is determined as *M. yokogawai* (see Saito, 1972; Shimazu, 1999). The other had five oral spines, large square penetration glands and the flame-cell formula of  $2[(3+3+3)+(3+3+3)]=36$ . This is regarded as *M. takahashii* (see Saito, 1972; Shimazu, 1999).

Saito & Shimizu (1968), Oyamada *et al.* (1996) and Shimazu (1999) recorded metacer-

cariae of *M. otsurui* from *G. castaneus*, *G. urotaenia* and *T. obscurus* from Lake Kasumigaura and from *G. castaneus* and *T. brevispinis* from Lake Ogawara. Both the lakes are brackish water. *Metagonimus otsurui* looks as if it were a brackish-water species. However, Saito *et al.* (1982) and the present study clearly indicate that the species is a freshwater species. Cercariae of the species might be capable of entering brackish water alive to infect gobiids there or might infect gobiids in fresh water when gobiids have migrated up into there from brackish water.

## References

- Oyamada, T., N. Kudo, T. Kitahara & Y. Takatou, 1996. *Metagonimus otsurui* metacercarial infection in gobiid fish (*Tridentiger brevispinis*) collected from Lake Ogawara in Aomori Prefecture, Japan. *Jpn. J. Parasitol.*, **45**: 275–279.
- Prince Akihito, 1987. [Japanese trident gobies.] In N. Mizuno & A. Goto (eds.), [Freshwater Fishes of Japan: Their Distribution, Variation and Speciation], pp. 167–178, Tokai Univ. Press, Tokyo. (In Japanese.)
- Saito, S., 1972. On the differences between *Metagonimus yokogawai* and *Metagonimus takahashii*. I. The morphological comparisons. *Jpn. J. Parasitol.*, **21**: 449–458. (In Japanese, with English summary.)
- Saito, S., 1999. [*Metagonimus*—Studies after 1960.] In M. Otsuru, S. Kamegai & S. Hayashi (chief eds.), [Progress of Medical Parasitology in Japan], **7**: 205–215, Meguro Parasitological Museum, Tokyo (In Japanese.)
- Saito, S., J.-y. Chai, K.-h. Kim, S.-h. Lee & H.-j. Rim, 1997. *Metagonimus miyatai* sp. nov. (Digenea: Heterophyidae), a new intestinal trematode transmitted by freshwater fishes in Japan and Korea. *Korean J. Parasitol.*, **35**: 223–232.
- Saito, S. & T. Shimizu, 1968. A new trematode, *Metagonimus otsurui* sp. nov. from the fresh-water fishes (Trematoda: Heterophyidae). *Jpn. J. Parasitol.*, **17**: 167–174.
- Saito, Y., T. Yamashita, T. Watanabe & S. Saito, 1982. Study on trematodes from Japanese water shrew VI. *Jpn. J. Parasitol.*, **31** (Suppl.): 60. (Abstract in Japanese.)
- Shimazu, T. 1999. *Metagonimus hakubaensis* sp. n. (Digenea, Heterophyidae) from Nagano, Japan: Morphology and life cycle. *Bull. Natn. Sci. Mus., Tokyo*, Ser. A, **25**: 87–99.