

Morphology of Metacercariae and Adults of *Metagonimus katsuradai* Izumi (Digenea, Heterophyidae) from Shiga, Japan

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Abstract Metacercariae of *Metagonimus katsuradai* Izumi, 1935 (Digenea, Heterophyidae) were found encysted chiefly in the scales and fin rays of *Tanakia lanceolata* (Temminck & Schlegel) and *T. limbata* (Temminck & Schlegel) (Osteichthyes, Cyprinidae) caught in inflowing waterways of Lake Biwa in Shiga Prefecture, Japan. They were experimentally fed to golden hamsters. Gravid adults were recovered from the small intestine of them. The metacercariae and adults are described. The flame-cell formula in the metacercarial stage and the ventrogenital sac and gonotyls in the adult stage are described for the first time.

Key words: *Metagonimus katsuradai*, Digenea, Heterophyidae, morphology, metacercariae, adults, freshwater fishes, golden hamster, Japan

Introduction

Izumi (1935a) found metacercariae of a species of the genus *Metagonimus* Katsurada, 1912 (Digenea, Heterophyidae) in *Acheilognathus lanceolata* [sic] *intermedia* [sic] (Temminck & Schlegel) [now *Tanakia lanceolata* (Temminck & Schlegel)], *Pseudorasbora parva* (Temminck & Schlegel) and *Zacco platypus* [sic, correctly *platypus*] (Temminck & Schlegel) (Osteichthyes, Cyprinidae) caught in the upper reaches of the Shoka River and vicinity in Hyogo Prefecture, Japan. He experimentally fed them to mice (species name not given), white mice (species name not given), a rat, a rabbit, a puppy and a kitten, and subsequently recovered adults from the animals. He described a new species, *M. katsuradai*, on the basis of the adults. He himself and his family ingested metacercarial cysts from *T. lanceolata*, and subsequently found eggs of *M. katsuradai* in their feces 6 days after infection on. Kurokawa (1939) found cercariae in *Melanaides* [sic] (*Semisulcospira*) *libertina* Gould [now *S. libertina* (Gould)] (Gastropoda, Pleuroceridae) collected in the Shoka River, experimentally exposed them to *P. parva* and *T. lanceolata* then,

and subsequently obtained metacercariae from the fishes. He experimentally fed the metacercariae to white mice, a puppy and a duck then, and subsequently recovered adults of *M. katsuradai* from the animals.

A few species of freshwater fish examined during a series of fish parasite workshops held at the Lake Biwa Museum (LBM), Kusatsu, Shiga Prefecture, had heavy black spots caused by a metacercarial infection in the skin. Metacercariae taken out of these fishes were experimentally fed to golden hamsters. Adults recovered from the golden hamsters were identified as *M. katsuradai*. In this paper I describe the morphology of the metacercariae and adults.

Materials and Methods

The fish species bearing the black spots were *Tanakia lanceolata* (Temminck & Schlegel) and *T. limbata* (Temminck & Schlegel) caught at Miyake, Moriyama, Shiga Prefecture, in an irrigation canal about 3 km upstream from its outlet, via the channelized Moriyama River, into Lake Biwa's south basin, on 27 October and 1 November 2000, 1 May 2001 and 19 October 2002; and

T. lanceolata caught at Nishiyama, Kinomoto, Shiga Prefecture, in the Yogo River, about 9 km from its mouth near the north end of Lake Biwa, on 26 April 2001.

The scales and fins of several specimens of *T. lanceolata* and *T. limbata* caught in the irrigation canal at Miyake on 27 October 2000 were digested in artificial gastric juice [1 g of pepsin (1:10,000) and 7 ml of conc. hydrochloric acid in 1,000 ml of 0.9% sodium chloride] at 37°C for 1 hour. Metacercariae were collected from the sediment of the digestion under a stereoscopic microscope. Metacercariae (not counted) from *T. lanceolata* and *T. limbata* were given orally to two golden hamsters, one host species each, by a stomach tube. The golden hamsters were sacrificed under excessive anesthesia by diethyl ether and examined for adult worms 15 days after infection.

Metacercarial worms were pressed out of the cysts. Excysted metacercarial worms and adult worms recovered from the golden hamsters were slightly flattened, fixed in 70% ethanol, stained with Grenacher's alum carmine and mounted in Canada balsam. Some excysted metacercarial worms were observed alive for study of the excretory system and uterus.

Drawings were made with the aid of a drawing tube, some with supplementary free-hand details drawn from living material. Measurements (in μm) given in the morphological description of

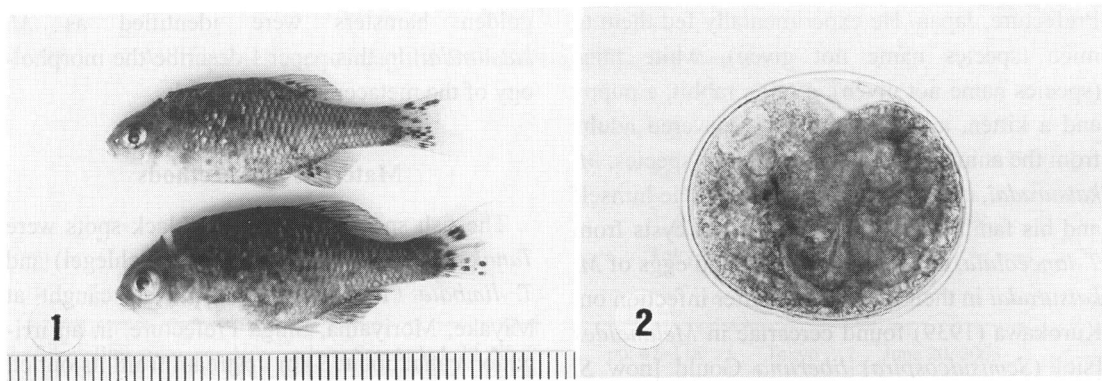
metacercarial cysts, excysted whole-mounted metacercariae and whole-mounted adults were taken from 10 specimens of each stage, all originating from *T. limbata*. The sucker ratio is the ratio of the width of the oral sucker to the length (or the longer diameter) of the ventral sucker. Representatives of the specimens studied have been deposited in the National Science Museum, Tokyo (NSMT) (specimen numbers: NSMT-PI 5223-5225) and in the LBM (accession numbers: LBM Misc. Inv. FY2002-2025). Two heavily spotted specimens of *T. limbata* caught at Miyake on 19 October 2002 also have been deposited in the LBM (registration number: 1210026804).

Results

Metacercariae

Heavy black spots, each surrounding a metacercarial cyst, were present in the connective tissue of the skin overlying the infected scales and fin rays of the fishes (Fig. 1). Large numbers of metacercariae were collected from both *T. lanceolata* and *T. limbata*. Only a minority of them showed movement within the cysts, with a distinctly visible excretory vesicle; but the majority were immotile, with an indistinct excretory vesicle.

Description (Figs. 2 and 3). Cyst (Fig. 2) broadly elliptical, flattened, 168–200 by 152–190; cyst wall thin, 2–4 thick.



Figs. 1–2. *Metagonimus katsuradai*. 1. *Tanakia limbata* bearing black spots caused by infection of metacercariae, caught at Miyake on 19 October 2002, scale unit=1 mm. 2. Metacercarial cyst from *T. limbata* caught at Miyake on 19 October 2002, living, cyst size 178 by 156 μm , cyst wall 4 μm thick.

Body (Fig. 3) ovate, spinose, 214–294 long by 130–160 wide; brown pigment deposited heavily in body. Transverse nerve commissure located posterodorsal to pharynx. Oral sucker subventral, 28–36 long by 28–44 wide. Prepharynx short. Pharynx 12–24 long by 14–22 wide. Esophagus 30–60 long, bifurcating at about junction of anterior and middle thirds of body. Intestinal ceca including many pale disks about 8–10 in diameter by 2 thick (not illustrated), extending posteriorly into post-testicular region, separated there. Ventral sucker internal to shoulder of right intestinal cecum, located at about mid-level of body, 22–28 long by 20–26 wide; sucker ratio 1:0.50–0.72. Testes slightly diagonal; anterior or left testis 22–38 long by 20–36 wide; posterior or right one 28–40 long by 18–36 wide. Ovary median, at about junction of middle and posterior thirds of body, 20–30 long by 20–32 wide. Posterior uterine loop embracing anterior testis. Excretory vesicle Y-shaped, lined with epithelial cells, including few excretory granules; epithelial cells large, scattered; 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; excretory pore posteroterminal.

Adults

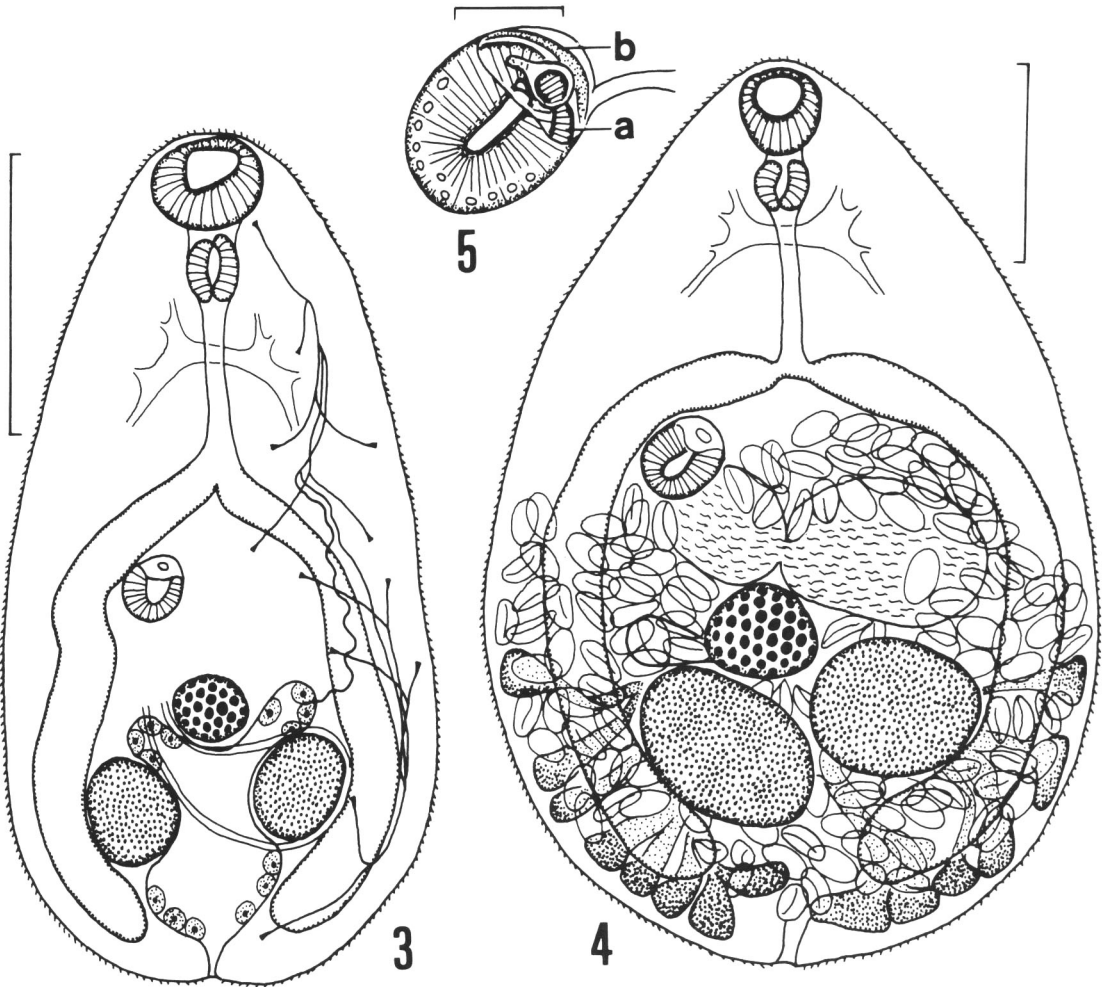
Adult worms were recovered from the middle third of the small intestine of the golden hamsters: 23 from the golden hamster fed metacercariae from *T. lanceolata*, and 21 from the other golden hamster fed metacercariae from *T. limbata*. All of them were fully gravid and morphologically very similar; and, therefore, they are regarded as belonging to a single species.

Description (Figs. 4 and 5). Body (Fig. 4) broad-oval, spinose, 390–530 long by 244–310 wide; brown pigment heavily deposited in body. Transverse nerve commissure located posterodorsal to pharynx. Oral sucker elliptical, subventral, 38–46 long by 42–50 wide. Prepharynx short. Pharynx elliptical, 26–30 long by 24–28 wide. Esophagus 64–114 long, bifurcating at about junction of anterior and middle thirds of

body. Intestinal ceca ventral, extending to mid-level of post-testicular region. Ventral sucker (Fig. 5) elliptical, small, 40–48 long by 32–40 wide, lying obliquely, internal to shoulder of right intestinal cecum, slightly posterior to cecal bifurcation, embedded in parenchyma, opening at anterior tip protruding into ventrogenital sac; sucker ratio 1:0.74–0.92. Ventrogenital sac small, thick-walled, anterosinistral to ventral sucker, internal to shoulder of right intestinal cecum. Gonotyls two; ventral one (Fig. 5, a) small, muscular, projecting from ventrosinistral base of ventrogenital sac, sometimes closing genital pore; dorsal one (Fig. 5, b) low, crescent, lying transversely at about mid-level of ventrogenital sac. Genital pore opening ventral to ventrogenital sac. Testes globular or elliptical, lying only slightly diagonally in about middle third of hindbody; anterior or left testis 60–110 long by 56–94 wide; posterior or right one 70–126 long by 64–94. Seminal vesicle bipartite, postbifurcal, 70–180 long by 28–80 wide. Ovary almost globular, median, located between seminal vesicle and testes, 30–50 by 50–80. Seminal receptacle retort-shaped, dorsal, posterodextral to ovary, anterior to posterior testis, 66–110 long by 40–64 wide. Uterus occupying all available space between cecal bifurcation and posterior ends of intestinal ceca, posterior loop embracing anterior testis. Eggs operculate, fully embryonated in distal part of uterus, 26–32 by 14–16 in balsam. Vitellaria dorsal, of branched clavate lobes, distributed between level of anterior border of anterior testis and posterior end of body, almost contiguous in post-testicular region.

Discussion

In morphology and measurements, the present adults agree on the whole with *M. katsuradai* as described by Izumi (1935a, b) and Kurokawa (1939). The seminal receptacle is located anterior not to the anterior testis (Izumi, 1935a, b; Kurokawa, 1939), but to the posterior testis (this paper). Izumi (1935a, b) and Kurokawa (1939) may have overlooked the seminal receptacle and



Figs. 3–5. *Metagonimus katsuradai*. 3. Excysted metacercarial worm, whole-mounted, excretory system on right side of body omitted, ventral view, scale bar=100 μm . 4. Gravid adult worm, recovered from small intestine of golden hamster 14 days after experimental infection, whole-mounted, ventral view, scale bar=100 μm . 5. Ventral sucker and ventrogenital sack, whole-mounted adult, ventral gonotyl (a) and dorsal gonotyl (b), ventral view, scale bar=25 μm .

mistaken the posterior portion of the bipartite seminal vesicle for the seminal receptacle. The vitellaria are not of irregularly shaped follicles (Izumi, 1935a, b; Kurokawa, 1939), but of branched clavate lobes (this paper). In the metacercarial stage, the intestinal ceca end posteriorly not at the level of the posterior border of the posterior testis (Izumi, 1935a, b; Kurokawa, 1939), but in the post-testicular region (this paper). The flame-cell formula in the metacercarial stage and the anatomical structure of the ventrogenital sac

and the position of the seminal receptacle in the adult stage are described herein for the first time.

In the adult stage, *M. katsuradai* is similar to *M. otsurui* Saito & Shimizu, 1968 as described by Saito & Shimizu (1968), Oyamada *et al.* (1996) and Shimazu & Urabe (2001) in having a small body, the oral sucker larger than the ventral sucker, the intestinal ceca and vitellaria both extending posteriorly into the post-testicular region, and the posterior uterine loop embracing the anterior testis. However, *M. katsuradai* is different

from *M. otsurui* in that the ovary and testes are more closely gathered together in the intercecal field of the middle third of the hindbody and in that the testes lie only slightly diagonally instead of distinctly diagonally. *Metagonimus katsuradai* also differs from *M. otsurui* in that the metacercarial cyst wall is thinner: 2–4 μm thick (Izumi, 1935a, b; this paper) instead of 7–18 μm thick (Saito & Shimizu, 1968; Oyamada *et al.*, 1996; Shimazu & Urabe, 2001). In the cercarial stage of *M. katsuradai*, the oral spines number six (Kurokawa, 1939) instead of four in *M. otsurui* (Shimazu & Urabe, 2001).

Two types of the flame-cell formula have been reported in the metacercarial stage of the genus *Metagonimus*, and accordingly are most probably present in the cercaria-metacercaria-adult generation: $2[(2+2+2)+(2+2+2)]=24$ in *M. katsuradai*, *M. otsurui*, *M. miyatai* Saito, Chai, Kim, Lee & Rim, 1997 and *M. hakubaensis* Shimazu, 1999; and $2[(3+3+3)+(3+3+3)]=36$ in *M. yokogawai* (Katsurada, 1912) Katsurada, 1912 and *M. takahashii* Suzuki in Takahashi, 1929 (see Saito, 1972; Shimazu, 1999, 2002; Shimazu & Urabe, 2001; this paper). It is not known what this difference means phylogenetically.

A natural first intermediate host of *M. katsuradai* is *Semisulcospira libertina* (Kurokawa, 1939). Natural and experimental second intermediate hosts are *Tanakia lanceolata*, *T. limbata*, *Pseudorasbora parva* and *Zacco platypus* (Izumi, 1935a, b; Kurokawa, 1939; this paper). Experimental final hosts include ducks, mice (species name not given), white mice (species name not given), rats, golden hamsters, rabbits, puppies, kittens and humans (Izumi, 1935a, b; Kurokawa, 1939; this paper).

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