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Metagonimus hakubaensis sp. n. (Digenea, Heterophyidae) from Nagano, Japan: Morphology and Life Cycle

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Abstract A new species, *Metagonimus hakubaensis* sp. n. (Digenea, Heterophyidae), is described and illustrated on the basis of adults recovered from the small intestine of rats which were experimentally fed metacercariae found in ammocoetes of a lamprey, *Lethenteron reissneri* (Dybowski) (Agnatha, Petromyzontidae), collected in a stream in Hakuba Village, Nagano Prefecture, central Japan. This new species is closely similar to *M. katsuradai* Izumi, 1935 and *M. otsurui* Saito et Shimizu, 1968 in that the oral sucker is larger than the ventral; but it is different from them in that neither the caeca nor the vitelline follicles extend posteriorly beyond the posterior testis, and the uterus hardly enters the post-testicular region. Mother and daughter rediae and cercariae of the new species are described from a prosobranch snail, *Semisulcospira dolorosa* (Gould) (Gastropoda, Pleuroceridae), from the same stream.

Key words: *Metagonimus hakubaensis* sp. n., Digenea, morphology, life cycle, Japan.

Members of a digenean genus, *Metagonimus* Katsurada, 1912 a (Heterophyidae), are intestinal parasites of mammals and birds in eastern and southeastern parts of Asia and in southeastern parts of Europe (Yamaguti, 1971). Metacercariae of a species of the genus were found encysted in ammocoetes of a lamprey in Hakuba Village, Nagano Prefecture, central Japan. In feeding experiments, they grew into adults in rats. Morphologically, the adults have proved to represent an undescribed species. Rediae and cercariae of the species were also obtained from a prosobranch snail in the same locality.

Materials and Methods

Ammocoetes of a lamprey, *Lethenteron reissneri* (Dybowski) (Agnatha, Petromyzontidae), and specimens of a prosobranch snail, *Semisulcospira dolorosa* (Gould) (Gastropoda, Pleuroceridae), were collected in a stream in Hakuba Village, Nagano Prefecture, central Japan, several times from June 1994 to June 1996 at irregular intervals. Ammocoetes were examined for metacercariae soon after collection. Some snails were examined for cercariae and rediae soon after collection, and some others were reared in the laboratory at room temperature until examination.

Ammocoetes were ground up in 0.9% saline in a mortar and filtered with a tea

strainer. The filtrate was washed in 0.9% saline a few times by the gravity sedimentation method. Metacercariae were collected from the last sediment under a binocular stereoscopic microscope. At the several initial examinations, ammocoetes were homogenized and digested in an artificial gastric juice at 37°C for 1–2 hours, but most metacercariae were found dead for unknown reason. Several smaller ammocoetes were fixed in cold 10% neutralized formalin, cut in serial sections (10 μm thick) and stained with Ehrlich's acid haematoxylin and eosin. Isolated metacercariae of undetermined numbers were given by stomach tube to seven rats, which were examined for adults 7 (one rat), 8 (one rat), 14 (two rats) and 22 (three rats) days later.

Metacercarial worms were excysted by pressure between slide glass and cover glass, slightly flattened, fixed in 70% ethanol and stained with alum carmine. Adults were slightly flattened, fixed with AFA and stained with Heidenhain's iron haematoxylin. Some 7-day-old adults were fixed in hot 5% neutralized formalin and stained with Ehrlich's acid haematoxylin. Rediae and cercariae were obtained from crushed snails. Mother rediae were slightly flattened, fixed in 70% ethanol and stained with alum carmine. Daughter rediae and cercariae were fixed in hot 5% neutralized formalin and stained with Ehrlich's acid haematoxylin. Some others were slightly flattened, fixed in Zenker-formol and stained with 0.5% toluidine blue. A few infected snails were fixed in cold Zenker-formol, cut in serial sections (7 μm thick) and stained either with haematoxylin and eosin or with 0.5% toluidine blue. All the stained specimens were mounted in Canada balsam. The excretory system was observed on both live and stained materials. Neutral red and methylene blue were used as vital stains.

For use in an experimental infection of cercariae to ammocoetes, 16 ammocoetes were caught in the River Chikuma in Saku, Nagano Prefecture, in December 1995. Seven (40–130 mm in total body length) were kept in an aquarium together with snails collected in the stream in October 1995 at 16°C for 5 months and then examined for metacercariae. Cercariae were found swimming in the water of the aquarium on the beginning and last days of the experiment. As control, the remaining nine ammocoetes (about 150 mm) were examined for metacercariae on the day after the capture, but all were negative for *Metagonimus* metacercariae.

The type and voucher specimens of *M. otsurui* Saito et Shimizu, 1968 were borrowed from the Meguro Parasitological Museum, Tokyo (MPM) (MPM Coll. No. 19157, holotype and 11 paratypes); and from Dr. S. Saito, Yamagata University School of Medicine, Yamagata (39 paratypes, 119 voucher adult specimens and 6 excysted metacercarial worms).

Measurements are given in micrometres 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. Drawings were made with the aid of a drawing tube and supplements with free-hand details from living material. The type series and representative voucher specimens have been deposited in the National Science Museum, Tokyo (NSMT) (NSMT-Pl 4552–4563); and in the Institute of Parasitology, Academy of

Sciences of the Czech Republic, České Budějovice, Czech Republic (IPCAS) (IPCAS D-416).

Results

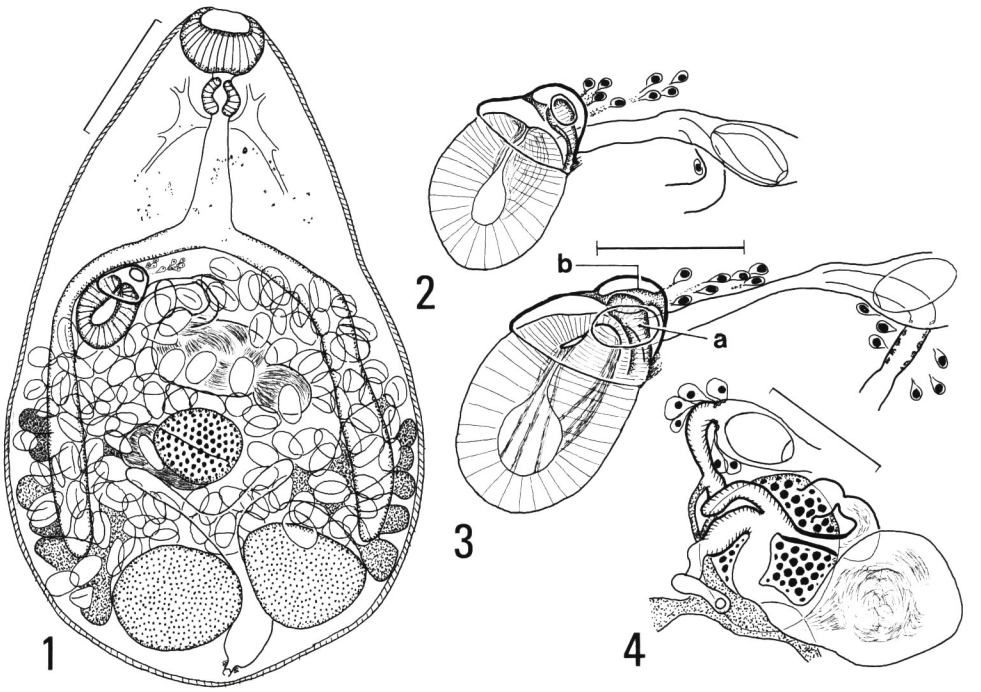
Adults

A total of 63, 63, 9 and 58 fully gravid adults were recovered from the posterior half of the small intestine of the rats 7, 8, 14 and 22 days post infection, respectively. The following is a description of the holotype and 57 paratypes (22-day-old flattened adult specimens), with measurements of 20 specimens including the holotype (those of the holotype in parentheses).

Metagonimus hakubaensis sp. n.

(Figs. 1–4)

Description. Body oval, 490–640 (487) long by 280–420 (312) wide (Fig. 1). Tegument covered with scales. Eyespot pigment dispersed in forebody. Brown body pigmentation not heavy. Transverse nerve commissure located postero-dorsal or dorsal to pharynx. Oral sucker elliptical, subterminal, 43–58 (50) long by 58–78 (62) wide. Prepharynx very short. Pharynx elliptical, 27–35 (28) long by 19–31 (28) wide. Oesophagus short, 20–94 (94) long, bifurcating into two caeca at about junction of anterior and middle thirds of body. Caeca ventral, short, ending posteriorly at mid-level of posterior testis; right caecum longer than left. Ventral sucker elliptical, lying obliquely, submedian, just medial to right caecal flexure, embedded in parenchyma, opening at anterior tip of its anterior part protruded into ventrogenital sac (Figs. 2–3), usually smaller than, or rarely as large as or slightly larger than, oral sucker, 51–70 (54) long by 39–51 (42) wide; sucker ratio 1 : 0.75–1.06 (1 : 0.87). Ventrogenital sac large, thick-walled, antero-sinistral to ventral sucker, opening postero-medial to shoulder of right caecum, accompanied by several gland cells. Gonotyls two; ventral one (Fig. 3, a) tall, muscular, projecting from ventro-sinistral base of ventrogenital sac; dorsal (Fig. 3, b) short, crescent, including very fine granules, lying transversely at about mid-level of ventrogenital sac. Testes globular or oval, diagonal in posterior-most part of body; anterior or left testis 100–150 (90) long by 70–120 (80) wide; posterior or right 100–160 (100) long by 80–120 (80) wide. Seminal vesicle bipartite, posterior to intestinal bifurcation, 80–136 (110) long by 40–64 (60) wide. Pars prostatica small, with prostatic cells 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 (Figs. 2–3). Ovary almost globular, median, between seminal vesicle and testes, 60–90 (60) long by 70–100 (70) wide. Oviduct short; Laurer's canal short; ootype-complex antero-sinistral to ovary (Fig. 4). Seminal receptacle sinistral, postero-dorsal or dextral to



Figs. 1–4. *Metagonimus hakubaensis* sp. n., adults from experimentally infected rats. —— 1. Entire worm, holotype, ventral view. Scale bar=100 μm. 2. Terminal genitalia, holotype, ventral view. Scale bar=50 μm. 3. Terminal genitalia, paratype, ventral (a) and dorsal (b) gonotyls, ventral view. Scale bar=50 μm. 4. Ootype-complex, paratype, dorsal view. Scale bar=50 μm.

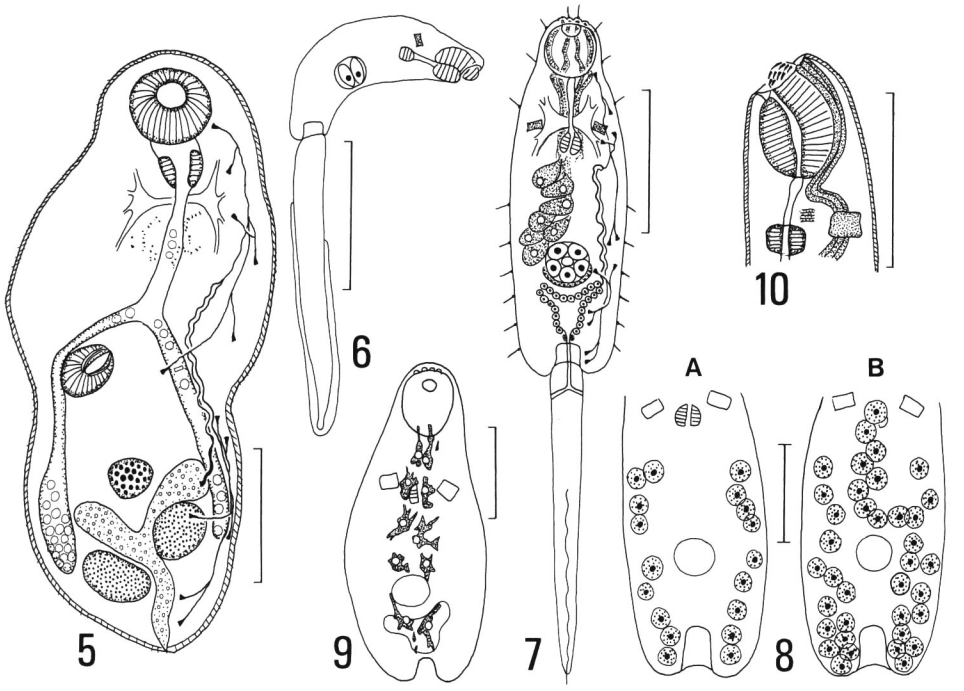
ovary, 60–160 (90) long by 40–100 (40) wide. Uterus occupying all available space between intestinal bifurcation and testes, rarely extending posteriorly between two testes; metraterm short. Eggs operculate, fully embryonated when laid, 28–34 by 16–20 (28–32 by 16–20) in balsam. Vitelline follicles dorsal, clavate, distributed between level of anterior border of ovary and mid-level of testicular region; vitelline reservoir postero-dorsal to ovary. Excretory vesicle ventral, Y-shaped, bifurcating posterior to ovary, with short arms; excretory pore terminal.

Host. Rat (experimental).

Site of infection. Small intestine.

Type specimens. Holotype and 48 paratypes (NSMT-Pl 4552) and 9 paratypes (IPCAS D-416) (flattened 22-day-old adult specimens).

Additional notes on adults. In flattened 7-day-old adult specimens (NSMT-Pl 4553), the body was oval, measuring 354–510 long by 216–280 wide; the sucker width ratio was 1 : 0.72–0.93; and eggs were 26–36 by 14–19. In hot formalin-fixed 7-day-old adult specimens (NSMT-Pl 4553), the body was pyriform, measuring 320–380 long by 170–202 wide; the sucker width ratio was 1 : 0.89–1.07; and eggs were

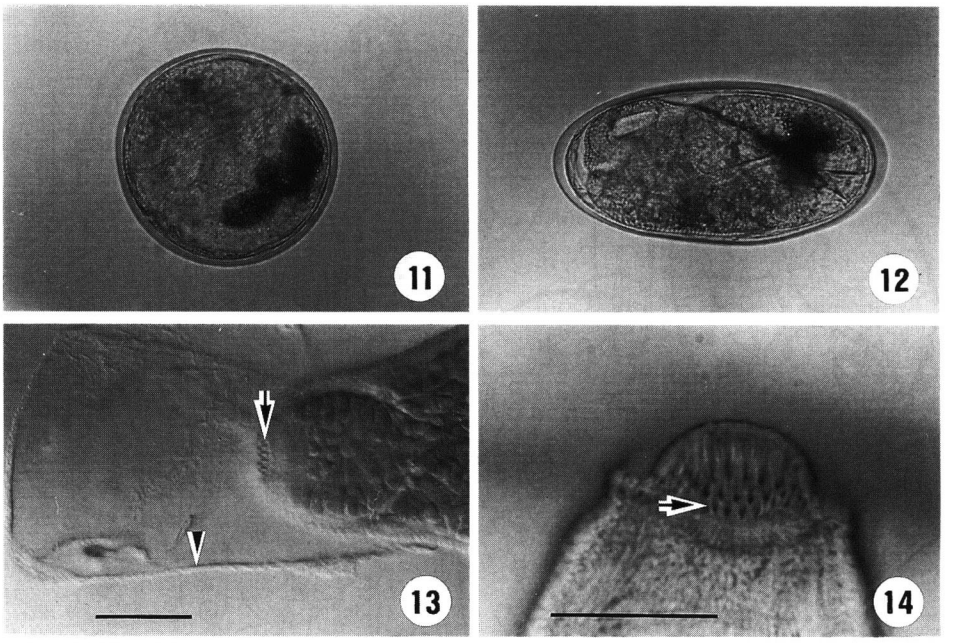


Figs. 5–10. *Metagominus hakubaensis* sp. n. —— 5. Excysted metacercarial worm from a naturally infected ammocoetes, living, flame cells and excretory canals on right side omitted, ventral view. Scale bar=100 μm. 6–10. Cercariae from naturally infected *Semisulcospira dolorosa*. 6. Entire worm, hot formalin fixed, lateral view. Scale bar=100 μm. 7. Entire body, living, penetration glands on left side and flame cells and excretory canals on right side omitted, ventral view. Scale bar = 100 μm. 8. Body proper, showing cystogenous glands on ventral (A) and dorsal (B) sides of body. Scale bar=50 μm. 9. Body proper, showing mucoid glands, ventral view. Scale bar=50 μm. 10. Anterior part of body proper, hot formalin fixed, lateral view. Scale bar=50 μm.

26–30 by 16–18. Internal organs were slightly larger in the former than in the latter. The flame-cell formula was 2 [(2+2+2)+(2+2+2)]=24 in several of live 7-day-old adults. Fully-embryonated uterine eggs measured 27–35 by 16–18 in live 14-day-old adults.

Metacercariae from naturally infected hosts

All of 40 ammocoetes (105–135 mm in total body length) caught on 1 June 1995 harboured metacercariae. The intensity of infection of metacercariae per fish was not counted. In the serial sections of the ammocoetes, metacercarial cysts were found much more in the walls of the pharynx, oesophagus and gill pouches, more in the kidney and liver, less in the walls of the oral hood, oral cavity and intestine, and



Figs. 11–14. *Metagonimus hakubaensis* sp. n. —— 11–12. Metacercariae from naturally infected ammocoetes. 11. Globular cyst, living, cyst 160 μm in diameter and 7 μm thick. 12. Elliptical cyst, living, cyst 242 by 114 μm and 4–10 μm thick. 13. Metacercaria from an experimentally infected ammocoetes, living, showing four cercarial oral spines in posterior row (arrow) and thin cyst wall less than 2 μm thick (arrowhead), ventral view. Scale bar=20 μm. 14. Cercaria from a naturally infected *Semisulcospira dolorosa*, living, showing four oral spines (arrow) in posterior row, ventral view. Scale bar=20 μm.

much less in the peritoneum, heart, parietal muscles and dermis. The morphology of the metacercariae was as follows, with measurements of ten excysted, flattened worms.

Description (Figs. 5, 11–12). Cysts globular, 133–179 in diameter (Fig. 11); or elliptical, 129–152 by 144–211 (Fig. 12), somewhat flattened; cyst wall transparent, 5–10 thick, composed of a very thin inner and a thick outer layer, sometimes surrounded by a rough tissue possibly of host origin.

Metacercarial worms similar in general morphology to adults (Fig. 5). Body elongate, 273–390 long by 144–156 wide. Tegument covered with scales. Brown body pigmentation not heavy. Oral sucker 50–70 long by 52–78 wide. Prepharynx very short. Pharynx 22–36 long by 20–32 wide. Oesophagus long, bifurcating into two caeca slightly anteriorly to mid-level of body. Caeca short, terminating at mid-level of testicular region, including transparent disks 6–8 in diameter. Ventral sucker 30–46 long by 28–36 wide; sucker ratio 1 : 0.50–0.65. Anterior testis 23–47 long by 23–39 wide; posterior 27–55 long by 23–55 wide. Ovary 19–35 long by 27–39 wide.

Uterus not clearly observed. Excretory vesicle filled with fine granules less than 4 in diameter; main excretory canals connected subterminally to respective arms of excretory vesicle, ciliated in short posteriormost part; flame-cell formula 2 [(2+2+2)+(2+2+2)]=24.

Metacercariae from experimentally infected hosts

A total of 15 encysted metacercariae were obtained from undetermined sites of the seven experimentally infected ammocoetes. In seven of them, the cysts measured 104–132 by 80–125, with a thin wall 1–3 thick; two eyespots were present; four cercarial oral spines were observed in the posterior row (Fig. 13); the caecal lumens were formed; the ventral sucker was located either still median or medial to the right caecal flexure. In eight others, the cysts measured 144–171 by 129–156, with a thick wall 6–8 thick; the eyespot pigment was dispersed; no cercarial oral spines were observed; and the ventral sucker was medial to the right caecal flexure. Their worms were similar in general morphology to the above-described metacercarial ones from naturally-infected hosts.

Cercariae

In 1995, 15 of 162 snails (13–40 mm in shell height) collected on 1 June and 18 of 250 (16–54 mm) collected on 9 and 16 September were infected with developing rediae and cercariae. Fully-formed cercariae emerged naturally from some snails whenever snails were examined. It was found that (1) cercariae escaped daughter rediae while still at early stages of development, in which a pair of eyespots were formed, the penetration glands were weakly developed, and the flame-cells were arranged in a formula of usually 2 [(1+1+1)+(1+1+1)]=12 or rarely 2 [(2+1+1)+(1+1+1)]=14; and (2) after having fully developed in the host, they left the host by an unknown route. The morphology of fully-formed cercariae was as follows, with measurements of ten cercariae fixed in hot formalin.

Description (Figs. 6–10, 14). Of pleurolophocercous type (Figs. 6–7). Body elongate, finely spinose, 180–210 long by 54–70 wide; at least 10 pairs of sensory hairs seen on lateral body margins. Brown body pigmentation not heavy. Tail inserted deeply in tail socket, 250–290 long by 22–26 wide; finfold beginning at about mid-level ventrally and at end of anterior third of tail dorsally, 10 high. A pair of eyespots dorsal, 10–12 by 6–10, present at level of cerebral ganglia. Paired cerebral ganglia present dorso-lateral to prepharynx, with a transverse commissure dorsal to prepharynx. Penetration glands consisting of anterior 3 and posterior 4 pairs, elliptical, 10–16 by 8–12, all similar in texture, lying along median line between pharynx and mid-level 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 (Figs. 7, 10). Cystogenous glands round, large,

present between pharynx and posterior end of body, numbering 18 on ventral and 38 on dorsal side of body (Figs. 8 A–B). Mucoid glands lying along median line in ventral parenchyma, consisting of 5 pairs (4 pairs between oral sucker and ventral sucker and 1 pair posterior to ventral sucker) (Fig. 9). Oral sucker elliptical, subterminal, 34–42 long by 28–32 wide; oral spines arranged in 3 transverse rows on apparently naked dorsal lip surmounting subventral opening of mouth, numbering 4 in posterior row, about 11 in middle and about 13 in anterior (Figs. 10, 14). Prepharynx long, 12–34 long. Pharynx elliptical, 12–14 long by 12–16 wide, posterior to nerve commissure. Oesophagus bifurcating about halfway between pharynx and genital primordium; caeca sometimes visible as faint lines of cells terminating in front of arms of excretory vesicle. Ventral sucker globular, median, 28–30 in diameter, at about junction of middle and posterior thirds of body; at least 5 large cells but no muscle fibres seen between inner and outer lamellae. Genital primordium dorsal to ventral sucker, 20–28 in diameter. Excretory vesicle Y-shaped, epithelial, thick-walled, with short arms; main collecting canals connected subterminally to respective arms of excretory vesicle, not ciliated in posteriormost parts; common excretory canal extending short distance into tail, bifurcating to open outside with two lateral pores; flame-cell formula 2 [(2+2+2)+(2+2+2)]=24.

Daughter rediae

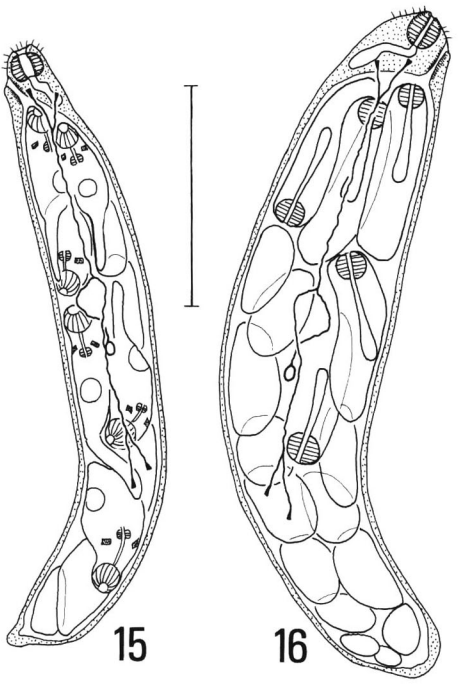
Daughter rediae in various developing stages were found around the alimentary canal and deeper parts of the body of snails; and larger ones were seen mostly in the mid-gut gland. The morphology of larger daughter rediae was as follows, with measurements of ten fixed in hot formalin.

Description (Fig. 15). Body elongate, 390–624 long by 86–132 wide, containing many developing cercariae (3–7 of them with eyespots) in body cavity, bearing sensory hairs of undetermined number around mouth at anterior tip of body. Pharynx almost spherical, 28–34 long by 30–34 wide. Caecum short, 30–80 long, extended or S-shaped. Birth pore lateral at mid-level of pharynx, with a short canal leading to body cavity. Flame-cell formula 2 [(1+1)+(1+1)]=8; excretory pores at about mid-level of body.

Mother rediae

A small number of large mother rediae were found among developing daughter rediae around the alimentary canal of one snail that was examined 3 weeks after collection on 9 September 1995. A large number of mother rediae were obtained together with developing mother or daughter rediae or both from around the alimentary canal of one snail that was examined 3 months after collection on 1 June 1995. Attempts to find the sporocyst stage in the two snails failed. The morphology and measurements of ten, slightly flattened, larger mother rediae were as follows.

Description (Fig. 16). Body similar to that of daughter rediae, 409–702 long



Figs. 15–16. *Metagonimus hakubaensis* sp. n., daughter and mother rediae from naturally infected *Semisulcospira dolorosa*. 15. Daughter redia, hot formalin fixed. Scale bar=200 μm. 16. Mother redia, flattened, living. Scale bar=200 μm.

by 109–156 wide, containing many developing daughter rediae. Pharynx 30–36 by 26–30. Caecum 40–70 long. Flame-cell formula 2 [(1+1)+(1+1)]=8.

Discussion

Katsurada (1912a) erected a new genus, *Metagonimus*, to contain *Heterophyes yokogawai* Katsurada, 1912 as the type and only species. The genera *Loxotrema* Kobayashi, 1912 (preoccupied), *Yokogawa* Leiper, 1913 and *Loossia* Ciurea, 1915 are its synonyms (Katsurada, 1912b; Ciurea, 1924; Poche, 1926; Faust and Nishigori, 1926; Witenberg, 1929). The following six species have so far been placed in the genus: *M. yokogawai* (Katsurada, 1912) (=*H. yokogawai*; *L. ovatum* Kobayashi, 1912; *Y. yokogawai* Leiper, 1913; *M. ovatus* Yokogawa, 1913b) from Taiwan and Japan; *M. romanicus* (Ciurea, 1915) [=*L. romanica* Ciurea, 1915; *L. parva* Ciurea, 1915; *M. parva* (Ciurea, 1915); *L. dobrogiensis* Ciurea, 1915; *M. dobrogiensis* (Ciurea, 1915)] from Romania; *M. takahashii* Suzuki in Takahashi, 1929 from Japan; *M. minutus* Yokogawa in Katsuta, 1932 from Taiwan; *M. katsuradai* Izumi, 1935 from Japan; and *M. otsurui* Saito et Shimizu, 1968 from Japan (Katsurada, 1912a, b;

Ciurea, 1915, 1924; Ransom, 1920; Poche, 1926; Faust and Nishigori, 1926; Takahashi, 1929; Witenberg, 1929; Katsuta, 1932; Izumi, 1935; Saito and Shimizu, 1968; Yamaguti, 1971). For a review of the Japanese species, see Ito (1964).

The oral sucker was usually larger than the ventral in the adults of the present species. In this respect, the species is similar to *M. katsuradai* as described by Izumi (1935) and Kurokawa (1939) and *M. otsurui* as described by Saito and Shimizu (1968) and Oyamada *et al.* (1996). However, the species is different from both of the latter in that (1) the caeca are shorter, ending posteriorly at the mid-level of the posterior testis instead of entering the post-testicular region; (2) the vitelline follicles are distributed posteriorly only to the mid-level of the posterior testis instead of to the posterior end of the body; and possibly (3) the uterus is coiled usually pre-testicularly but rarely extends posteriorly between the two testes instead of always embraces the anterior testis in *M. otsurui* and extends posteriorly between the two testes in *M. katsuradai*. My re-examination of the original specimens of *M. otsurui* has confirmed that Saito and Shimizu (1968) correctly described the posterior extents of the caeca and vitelline follicles and the distribution of the uterus. No specimens of *M. katsuradai* have been available to me for re-examination. On the other hand, the present species is distinguished from *M. yokogawai*, *M. romanicus*, *M. takahashii* and *M. minutus* because the oral sucker is distinctly smaller than the ventral in all of the latter (Yokogawa, 1912, 1913a, b; Kobayashi, 1912; Leiper, 1913; Ciurea, 1915; Takahashi, 1929; Suzuki, 1930; Katsuta, 1932; Ito, 1964). Consequently, the present species differs in adult morphology from all the known species; and a new species, *Metagonimus hakubaensis* sp. n., is proposed for it. This new species is characterized mainly by that (1) the body is small, less than 700 μm in length; (2) the oral sucker is usually larger than the ventral sucker, with the sucker ratio being 1 : 0.72–1.07; (3) the caeca are short, ending posteriorly at the mid-level of the posterior testis; (4) the uterus is usually pre-testicular but rarely extends posteriorly between the two testes; (5) the vitelline follicles are distributed between the level of the anterior border of the ovary and the mid-level of the posterior testis; and (6) eggs measure 26–36 by 14–19 μm. The first characteristic might be one of host-induced variations.

In the exposure experiment, a total of 15 metacercariae were recovered from the seven experimentally-infected ammocoetes, but no *Metagonimus* metacercariae were found in the nine control ammocoetes. In the seven of the 15 metacercariae, the cysts were smaller and thinner than those of the remaining eight; two eyespots and four cercarial oral spines were observed (Fig. 13); and the ventral sucker was either still median or medial to the right caecal flexure. The eight had dispersed eyespot pigment but no cercarial oral spines, and they resembled those from the naturally infected hosts in size and thickness of the cyst and in general morphology of the worm. Moreover, the present cercaria was the only pleurolophocercous cercaria found in the snails from the stream in Hakuba Village. It seems certain that all the 15 metacercariae were of experimental infection, which means that the present cercaria belongs to

the new species. The seven metacercariae should have recently invaded the hosts. According to Takahashi (1929), the oral spines disappear within 6 to 8 days after cercariae of *M. takahashii* enter the second intermediate host.

In addition to ammocoetes of *L. reissneri*, *Misgurnus anguillicaudatus* (Cantor), *Cobitis biwae* Jordan et Snyder (Cobitidae), *Salvelinus leucomaenis pluvius* (Hilgendorf), *Oncorhynchus mykiss* (Walbaum), *O. masou masou* (Brevoort), *O. masou ishikawae* Jordan et McGregor (or *O. masou masou*×*O. masou ishikawae*) (Salmonidae), *Phoxinus lagowskii steindachneri* Sauvage and *Carassius auratus langsdorfii* Cuvier et Valenciennes (Cyprinidae) were caught in the stream and examined for metacercariae. A few metacercariae of the new species were obtained only from undetermined sites of *M. anguillicaudatus* and *C. biwae* as well (unpubl. data). No gobiids live in the stream. Furthermore, several experimental attempts to infect *Chaenogobius urotaenia* (Hilgendorf) and *Rhinogobius* sp. (Gobiidae) with cercariae of the new species failed (unpubl. data). As for the life cycle, the new species utilizes *S. dolorosa* and ammocoetes of *L. reissneri*, *M. anguillicaudatus* and *C. biwae* as the first and the second intermediate host, respectively, in the stream in Hakuba Village. A natural definitive host is unknown. Metacercariae which were very similar to those of the new species were found in ammocoetes of *L. reissneri* from Lake Ogawara, Aomori Prefecture, Japan, in May 1997 (unpubl. data, NSMT-Pl 4564).

The metacercariae of the new species were found much more in the walls of the alimentary canal and gill pouches than in the dermis and parietal muscles of the ammocoetes. Ammocoetes of *L. reissneri* are burrowers in soft muddy bottoms and strain out organic matter as food at the mud-water interface. Most presumably, free-living cercariae of the new species after emergence from host snails infect ammocoetes by being passively swallowed by ammocoetes along with the organic matter or respiratory water which irrigates the gill pouches rather than by directly penetrating the skin.

The flame-cell formula was 2 [(1+1)+(1+1)]=8 in the mother and daughter rediae (Figs. 15–16) and 2 [(2+2+2)+(2+2+2)]=24 in the cercaria-metacercaria-adult generation (Figs. 5, 7) of the new species. The same formula was observed in metacercariae of *M. otsurui* obtained from *Chaenogobius castaneus* (O'Shaughnessy) and *Tridentiger brevispinis* Katsuyama, Arai et Nakamura (Gobiidae) from Lake Ogawara, Aomori Prefecture, in May 1997 (unpubl. data, NSMT-Pl 4565–4566). In *M. yokogawai* and *M. takahashii*, the flame-cell formula is 2 [(1+1)+(1+1)]=8 in daughter rediae (unpubl. data) and 2 [(3+3+3)+(3+3+3)]=36 in cercariae (Saito, 1972; unpubl. data). It was 2 [(3+3+3)+(3+3+3)]=36 in metacercariae and young adults of *M. takahashii* (unpubl. data). Therefore, there have so far been reported two different flame-cell formulae in the cercarial stage of the genus *Metagonimus*: 2 [(2+2+2)+(2+2+2)]=24 in the new species and possibly *M. otsurui* and 2 [(3+3+3)+(3+3+3)]=36 in *M. yokogawai* and *M. takahashii*.

The oral spines in the posterior row was four in the cercaria of the new species

(Fig. 14). They are four, five and six in *M. yokogawai*, *M. takahashii* and *M. katsuradai*, respectively (Takahashi, 1929; Kurokawa, 1939; Saito, 1972). The mucoid glands were composed of five pairs in the cercaria of the new species (Fig. 9). They are the same in number and arrangement as in *M. yokogawai* as observed by Ito and Watanabe (1958) and in *M. takahashii* (unpubl. data).

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