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Mayfly Larvae, *Ephemera japonica*, as Natural Intermediate Hosts of Salmonid Nematodes, *Sterliadochona ephemeridarum* and *Rhabdochona oncorhynchi*, in Japan

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Abstract

Larvae of freshwater salmonid nematodes, *Sterliadochona ephemeridarum* (third-stage) (Spirurida: Habronematoidea: Cystidicolidae) and *Rhabdochona oncorhynchi* (third- and fourth-stage) (Spirurida: Thelazioidea: Rhabdochonidae), were described from mayfly larvae, *Ephemera japonica*, collected in mountain rivers in central Japan. *E. japonica* larvae proved natural intermediate hosts for these two nematode species for the first time. Changes in number and shape of teeth of the prostom during development from the third-stage larva to the adult in *R. oncorhynchi* were briefly discussed.

Key words: nematode larvae; *Sterliadochona ephemeridarum*; *Rhabdochona oncorhynchi*; *Ephemera japonica* larvae; life cycles; Japan.

Sterliadochona ephemeridarum (Linstow, 1872) Petter, 1984 [= *Cystidicoloides ephemeridarum* (Linstow, 1872) Moravec, 1981] (Spirurida: Habronematoidea: Cystidicolidae), is a common parasite of the stomach of freshwater salmonids in Europe, Asia including Japan, and North America (Nagasawa *et al.*, 1987, 1989; Moravec, 1994). This nematode uses mayflies as the intermediate host in Europe and North America (Moravec, 1994), but the intermediate host in Japan has remained unknown. *Rhabdochona oncorhynchi* (Fujita, 1921) Fujita, 1927 (Spirurida: Thelazioidea: Rhabdochonidae), is a common parasite of the intestine of freshwater salmonids in Japan and Far Eastern Russia (Nagasawa *et al.*, 1987, 1989; Moravec, 1994), but nothing has been known about the intermediate host.

In the course of a life-cycle study of a digenean, aquatic insects of several species were collected in mountain rivers in central Japan and examined for parasites. Larvae of the mayfly *Ephemera japonica* were found to harbor larvae of both *S. ephemeridarum* and *R. oncorhynchi*. This paper describes the nematode larvae obtained. For the taxonomy of the two nematode species, the reader may refer to Moravec *et al.* (1981), Petter (1984), Moravec and Nagasawa (1985), and Moravec (1994).

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Materials and Methods

Mayfly larvae, *Ephemera japonica* (Insecta: Ephemeroptera: Ephemeridae), were collected from sandy silt taken in the Samu River at Fujisawa on September 23, 1992, and the Ide River at Araya on September 24, 1995, both in Iiyama City; and the Nakakurozawa (a small river) in Hakuba Village on September 13 and November 4, 1994, all in Nagano Prefecture. They were slightly crushed fresh with pressure of a cover glass and examined for parasites under a stereoscopic microscope. Nematode larvae found were fixed in hot 5% formalin and stored in glycerin-alcohol. In order to determine sites of infection of nematode larvae, several infected mayfly larvae were fixed in cold 5% formalin and made into serial paraffin sections (7–10 μm thick), which were stained with Delafield's hematoxylin and eosin. Neither incidences nor intensities of infection of nematode larvae in mayfly larvae were recorded.

Measurements are given in millimeters. Representatives of the specimens studied have been deposited in the National Science Museum, Tokyo (NSMT).

Sterliadochona ephemeridarum
(Figs. 1, 3–6)

Nematode larvae were found roughly coiled free (Fig. 1) in the muscles, and rarely in the hemocoel, of the abdomen of mayfly larvae collected in the Samu River on September 23, 1992 (NSMT-As 2478), the Ide River on September 24, 1995 (NSMT-As 2479-2480), and the Nakakurozawa on September 13 and November 4, 1994 (NSMT-As 2481). They consisted of males and females and apparently belonged to the same developmental stage.

Description of third-stage larva. Based on 10 males and 6 females (Figs. 3–6). Body 3.43–4.90 in length, 0.05–0.09 in maximum width; cuticular striations prominent, dense. Deirids not seen. Tail short, round, 0.04–0.07 long (0.06–0.07 long in males, 0.04–0.05 long in females), with a terminal knoblike process. Stoma sclerotized, slender, 0.09–0.10 long, anteriorly dilated like a funnel; boundary between prostom and remaining portion of stoma indistinct. Nerve ring 0.15–0.17 from anterior end of body. Excretory pore 0.18–0.21 from anterior end of body; excretory gland situated a short distance behind nerve ring, duct 0.03–0.05 long, gland cell 0.05–0.08 long by 0.01–0.02 wide. Muscular esophagus 0.47–0.64 long; glandular 0.71–1.31 long, with an esophagointestinal valve. Intestine 1.93–3.20 long. Rectum 0.04–0.07 long, with 3 large anterior (2 lateral and 1 dorsal) and several small posterior rectal glands. In males, genital primodium extending from behind glandular esophagus to in front of cloaca. In females, genital primodium extending from glandular esophagus usually to near rectum or sometimes into tail; parts of vagina and vulva indistinct.

Discussion. These nematode larvae are closely similar in general morphology to the known third-stage larva of *S. ephemeridarum* (see Moravec, 1994). Moreover, fourth-stage larvae and adults which were identifiable with this nematode as described by Moravec and Nagasawa (1985) and Moravec (1994) were found in the stomach of *Salvelinus leucomaenis pluvius* (Salmonidae) caught in the Ide River and Nakakurozawa during this study (my unpublished data). All the present nematode larvae are regarded as third-stage larvae of *S. ephemeridarum*. There was no significant difference in measurements except in length of the tail between males and females.

This is the first finding of the natural intermediate host, *E. japonica* larvae, for *S. ephemeridarum* in Japan. Further detailed studies of the life cycle and ecology of this nematode in Japan are needed. Anderson (1992) and Moravec (1994) reviewed the life cycle of the nematode.

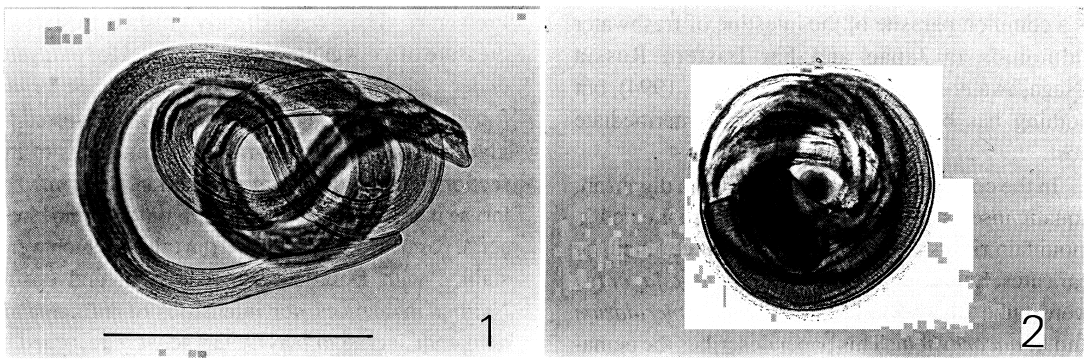
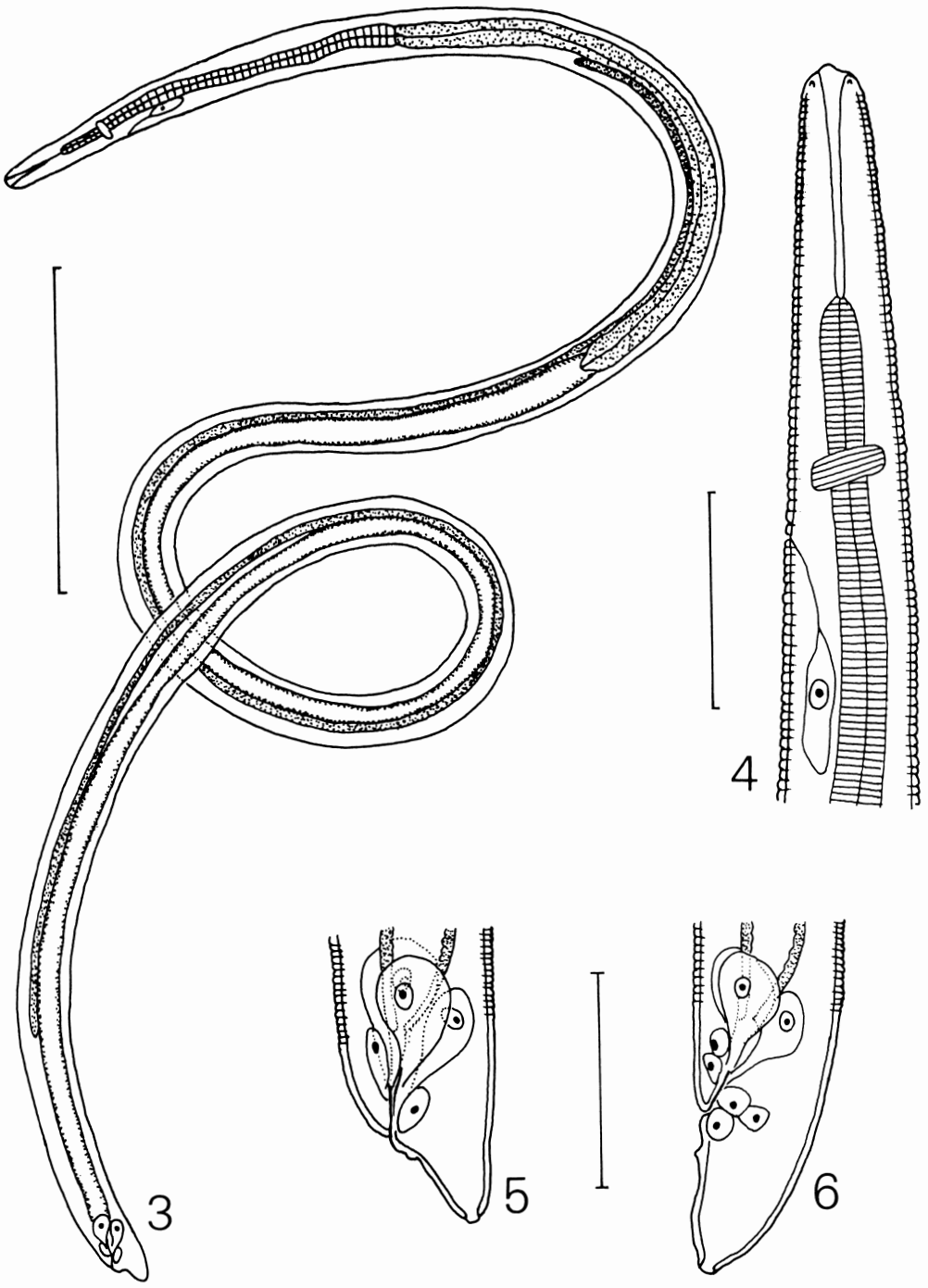


Fig. 1 A third-stage larva of *Sterliadochona ephemeridarum* from a formalin-preserved *Ephemera japonica* larva.
Fig. 2 A fourth-stage larva of *Rhabdochona oncorhynchi* from a formalin-preserved *E. japonica* larva.
(Scale bar: 0.5 mm in Figs. 1 and 2.)



Figs. 3–6 Third-stage larvae of *Sterliadochona ephemeridarum* from *Ephemera japonica* larvae, fixed in hot formalin.
Figs. 3–5 3: Entire body of a female, lateral view. 4: Anterior part of body, lateral view. 5: Posterior part of body, lateral view.
Fig. 6 Posterior part of a male, lateral view.
(Scale bars: 0.5 mm in Fig. 3; 0.1 mm in Figs. 4–6.)

Rhabdochona oncorhynchi
(Figs. 2, 7–10)

Nematode larvae were found spirally coiled individually in a thin-walled, hyaline capsule of host origin measuring 0.33–0.56 in diameter (Fig. 2) in the hemocoel, and rarely between the integument and the muscles, of the abdomen of mayfly larvae.

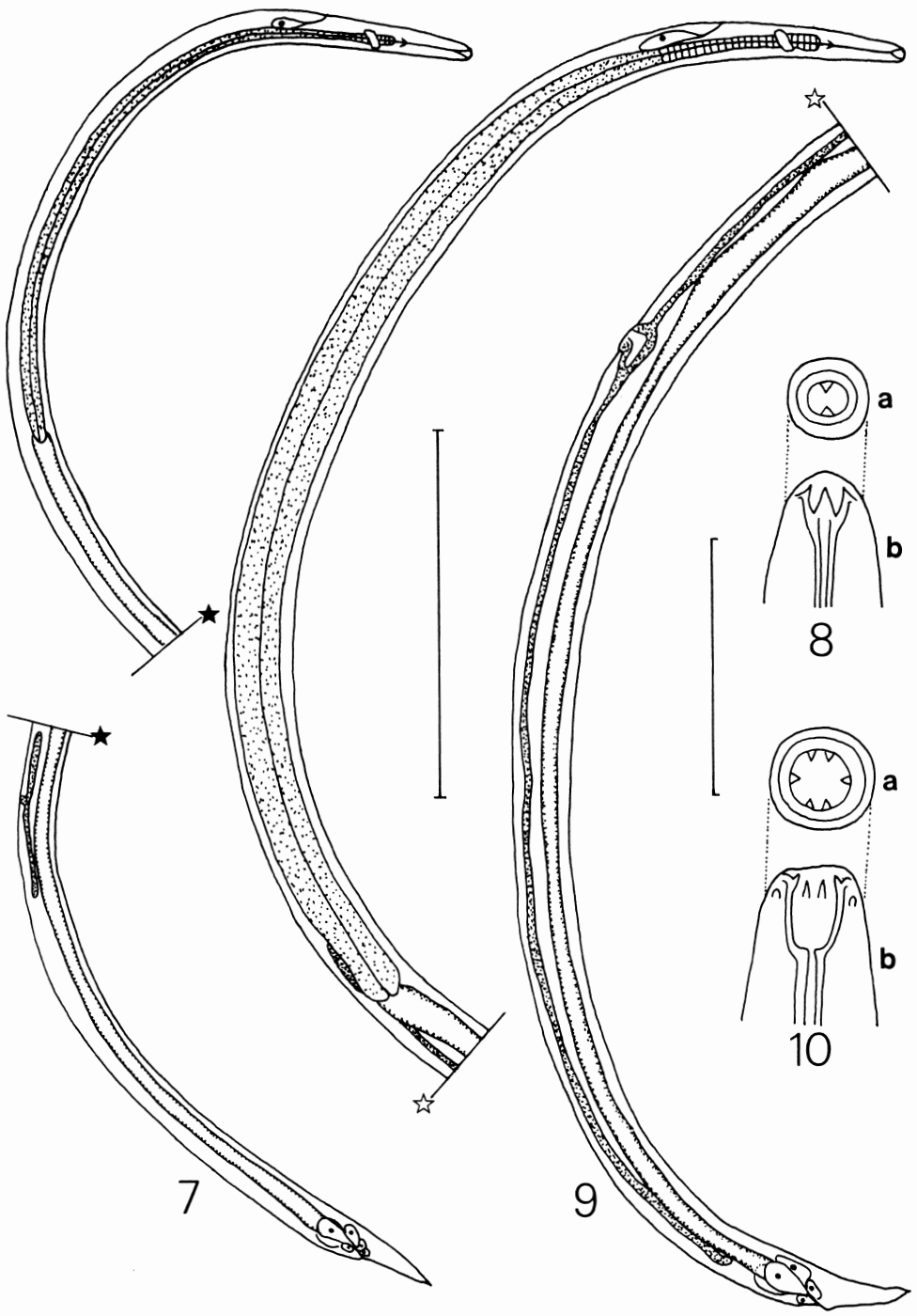
Many male and female larvae were obtained. They were classified into two groups according to body size and morphology: one female of group 1 from the Nakakurozawa on November 4, 1994; and many other males and females of group 2 from the Samu River on September 23, 1992 (NSMT-As 2478), the Ide River on September 24, 1995 (NSMT-As 2479 and 2482), and the Nakakurozawa on September 13 and November 4, 1994 (NSMT-As 2483).

Description of third-stage larva. Based on 1 female of group 1 (Figs. 7–8). Body 2.31 in length, 0.05 in maximum width; cuticle smooth. Deirids small, bifurcate, located at about junction of middle and posterior thirds of stoma. Tail conical, 0.10 long, with a sharp-pointed tip. Stoma sclerotized, slender, 0.10 long, anteriorly dilated to form a bell-shaped prostom; prostom about 0.01 long, having a pair of conical lateral teeth. Nerve ring 0.14 from anterior end of body. Excretory pore 0.20 from anterior end of body; excretory gland located slightly behind nerve ring, duct 0.03 long, gland cell 0.04 long by 0.02 wide. Muscular esophagus 0.15 long; glandular 0.77 long, with an esophagointestinal valve. Intestine 1.14 long. Rectum 0.02 long, with 3 large anterior (2 lateral and 1 dorsal) and at least 3 small posterior rectal glands. Genital primodium about 0.20 long; parts of vagina and vulva slightly anterior to midlevel of body.

Description of fourth-stage larva. Based on 10 males and 10 females of group 2 (Figs. 9–10). Body 2.75–4.60 in length, 0.07–0.11 in maximum width; cuticle smooth. Deirids small, bifurcate, located at about junction of middle and posterior thirds of stoma. Tail conical, 0.12–0.17 long. Oral opening round. Stoma sclerotized, slender, 0.09–0.12 long, anteriorly dilated to form a bell-shaped prostom; prostom 0.01–0.02 long by 0.01 wide, with 1 tooth on each of dorsal and ventral sides and 2 teeth on each lateral side; all teeth simple, sharp at tip. Nerve ring 0.13–0.17 from anterior end of body. Excretory pore 0.20–0.25 from anterior end of body; excretory gland situated a short distance behind nerve ring, duct about 0.02 long, gland cell 0.05–0.12 long by 0.01–0.02 wide. Muscular esophagus 0.15–0.22 long; glandular 1.21–2.07 long, with an esophagointestinal valve. Intestine 1.13–1.95 long. Rectum 0.05–0.08 long, with 3 large anterior (2 lateral and 1 dorsal) and at least 2 small posterior rectal glands. In males, testis extending anteriorly to junction of glandular esophagus and intestine to make a U-turn there; terminal genitalia weakly differentiated, leading to cloaca. In females, ovaries extending anteriorly to junction of glandular esophagus and intestine to make a U-turn there and posteriorly to near anus; vagina weakly formed; vulva located slightly posterior to midlevel of body, not yet opening outside.

Discussion. These two groups are different chiefly in body size and number of teeth of the prostom. The female of group 1 had one sharp tooth on each lateral side of the prostom, and the males and females of group 2 had two sharp teeth on each lateral side and one sharp tooth on each of the ventral and dorsal sides. In the number and arrangement of the teeth, the larvae of groups 1 and 2 agree to the third- and fourth-stage larvae, respectively, of *Rhabdochona ergensi* and *R. phoxini* (see Moravec, 1994). Moreover, fourth-stage larvae and adults which were identifiable with *R. oncorhynchi* as described by Moravec *et al.* (1981) and Moravec (1994) were found in the pyloric ceca and intestine of *S. leucomaenis pluvius* caught in the Ide River and Nakakurozawa during this study (my unpublished data). In morphology and measurements, the larvae of group 2 from mayfly larvae were identical with the fourth-stage larvae from *S. leucomaenis pluvius*. Although no feeding experiments have been made, it is concluded that the larvae of groups 1 and 2 from mayfly larvae are third- and fourth-stage larvae, respectively, of *R. oncorhynchi*. Males and females of the fourth stage did not differ significantly in measurements. The third-stage larva has already been lost.

This paper is the first to record *E. japonica* larvae as the natural intermediate host for *R. oncorhynchi*. This nematode needs further detailed studies of the life cycle and ecology. During this study, two fourth-stage female larvae of the nematode were obtained



Figs. 7–10 Larvae of *Rhabdochona oncorhynchi* from *Ephemera japonica* larvae, fixed in hot formalin.

Figs. 7 and 8 7: Entire body of a third-stage female larva, lateral view. 8: Head, apical view (a), lateral view (b), showing teeth of the prostom.

Figs. 9 and 10 9: Entire body of a fourth-stage female larva, lateral view. 10: Head, apical view (a), lateral view (b), showing teeth of the prostom.

(Scale bars: 0.5 mm in Figs. 7 and 9; 0.05 mm in Figs. 8 and 10.)

from the small intestine of a larva of the salamander *Onychodactylus japonicus* in the Nakakurozawa (my unpublished data). Anderson (1992) and Moravec (1994) reviewed life cycles of other *Rhabdochona* members. *Rhabdochona* nematodes are known to utilize not only larvae of mayflies but also, more rarely, larvae of stone flies (Insecta: Plecoptera) and caddis flies (Insecta: Tricoptera) (see also Moravec, 1995).

The adults of *R. oncorhynchi* from *S. leucomaenis pluvius* of the Ide River and Nakakurozawa possessed two bifurcated teeth on each lateral side of the prostom and three sharp teeth on each of the ventral and dorsal sides (my unpublished data; see also Moravec *et al.*, 1981; Ito *et al.*, 1987; Moravec, 1994). In this nematode at the localities examined, as in *R. ergensi* and *R. phoxini* (see Moravec, 1994), the teeth of the prostom are likely to change in number and shape through the third and fourth molts as follows: (1) on each lateral side, from one sharp tooth in the third-stage larva to two sharp teeth in the fourth-stage larva and then to two bifurcated teeth in the adult; and (2) on each of the ventral and dorsal sides, from none to one sharp tooth and then to three sharp teeth in the same order.

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