

Experimental Infection of Some Species of Freshwater Snails with *Clinostomum complanatum* (Trematoda: Clinostomatidae)

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Abstract

Four species of freshwater snails were experimentally exposed to *Clinostomum complanatum* miracidia. These snails were bred in water at 28±1°C and then crushed on the 21th–44th day after miracidial exposure. Furcocercous cercariae and rediae were removed from 10 out of 66 *Lymnaea japonica* and 8 out of 24 *Lymnaea ollula*, respectively. The morphological features and measurements of mature cercariae almost corresponded with the description on the *C. complanatum* cercaria by Lo *et al.* (1982). Both *L. japonica* and *L. ollula* were newly recorded as experimental snail hosts of *C. complanatum*.

Key words: cercaria, *Clinostomum complanatum*, experimental infection, *Lymnaea japonica*, *Lymnaea ollula*, snail host

Introduction

Clinostomum complanatum (Rudolphi, 1814) is a common parasite in Asia, Near East, Europe and America. In Japan, several species of fish and bird hosts of this fluke have been reported by Yamaguti (1938), Yoshino (1940), Kagei *et al.* (1984, 1988) and Aohagi *et al.* (1992a, b, 1993), since Yamaguti (1933) recorded it. In addition, 5 human cases caused by *C. complanatum* have been reported (Yamashita, 1938; Hori, 1942; Hirai *et al.*, 1987; Umezaki *et al.*, 1990; Yoshimura *et al.*, 1991). However, the information on the life cycle of this fluke is insufficient in Japan because the snail host has not been specified. The present study was therefore carried out in order to clarify the snail host of *C. complanatum*.

Materials and Methods

Parasite

The adult *C. complanatum* was obtained from

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the pharynx of grey herons, *Ardea cinerea* which were captured in the eastern part of Tottori Prefecture (see Aohagi *et al.*, 1992b). These flukes were put in 0.85% saline solution to obtain eggs. The laid eggs were incubated in water at 28±1°C. Spontaneously hatched miracidia on the 8th–10th day after incubation were used for the experiment.

Freshwater snails used

The snails used are as follows: 157 *Lymnaea japonica* (shell length [s.l.], 7.6–15.0mm), 84 *Lymnaea ollula* (s.l., 12.0–15.0mm), 70 *Physa acuta* (s.l., 5.2–8.0mm) and 40 *Gyraulus chinensis* (s.l., 4.2–6.0mm). Both *L. japonica* and *P. acuta* were originating from a ditch in Tottori City, Tottori Prefecture, Japan, and both *L. ollula* and *G. chinensis* from a rice field in Wakasa-cho, Yazu-gun, Tottori Prefecture, Japan. These snails were successively bred in the laboratory and only the laboratory-reared snails were used for the experiment.

Experimental infection

Five to ten snails were exposed to miracidia (3 miracidia per snail) at 28±1°C for 12 hours in the tissue culture dishes (3.0cm in diameter) containing 5ml of aged tap water.

Maintenance of snails

After the exposure, the snails were transferred

into the beakers (12.4cm in diameter) with about 1l of aged tap water and bred at $28\pm 1^\circ\text{C}$. The water in the beakers was changed every day. The snails were fed with a proper quantity of fish meal (Tetra-Fin[®], Tetra-Berke Co. Ltd.). These snails were crushed on the 21th–44th day after miracidial exposure and examined for larval flukes under a binocular dissecting microscope.

Morphological observations and measurements

The living larvae were used for the morphological observations. Some internal organs were observed by the aid of vital staining method using neutral red. Measurements were made on 20 specimens fixed in 10% hot formalin. All measurements are in μm .

Results

The larval flukes were removed from 10 out of 66 *L. japonica* and 8 out of 24 *L. ollula*, respectively (Table 1). Furcocercous cercariae and rediae were found in the midgut of each of the infected snails (Fig. 1). No larvae were detected from either 43 *P. acuta* or 36 *G. chinensis*.

The morphological features and measurements of mature cercariae and rediae removed from *L. japonica* are as follows.

Total length of cercaria measured 497–549 (Table 2). The body had a longitudinal dorsal fin and was covered with minute spines from the anterior one-fourth of the body to the end. The body measured 104–124 in length and 35–40 in width. The tail stem

and furcae were covered with the minute spines. The tail stem had 6 pair of long filamentary hairs and measured 294–319 in length and 21–27 in width. Each furca had a long filamentary hair, tapered toward ends and measured 76–107 in length. The alimentary tract was not well developed. The mouth opening was situated at the ventral side of the posterior one-third of the penetration organ. The pharyngeal structure was absent. The oesophagus extended posteriorly and ended in a dilated structure which existed in the middle of the body. The penetration organ was located at the anterior extremity of the body and measured 35–43 in length and 22–26 in width. The anterior tip of the penetration organ was covered with 9 rows of minute spines. Four pairs of penetration gland cells existed near a dilated structure. The penetration ducts extended anteriorly from the penetration gland cells and opened at the anterior top of the penetration organ with four small papillae. One pair of eye spots was located in the anterior part of the middle-third of the body and measured 4.5–6.3 in diameter. The ventral sucker analogue lay in the posterior one-third of the body. The genital primordium was not recognizable. The excretory bladder was elongated and located in the posterior end of the body. Two primary excretory ducts ran forward from the antero-lateral side of the excretory bladder. Each duct bifurcated into two secondary canals at the level of the anterior extremity of the ventral sucker analogue. Four pair of flame cells were arranged symmetrically in the body, and one pair near the anterior extremity of the tail stem. The flame cell formula was $2[(1+1)+(1+1+[1])]=10$.

Table 1 Results of experimental infection of 4 species of the freshwater snails with *Clinostomum complanatum* miracidia

Species of snail	No. of snails exposed to miracidia	No. of snails crushed between 21 and 44 days postexposure	No. of snails infected with <i>C. complanatum</i> larvae
<i>Lymnaea japonica</i>	157	66	10
<i>Lymnaea ollula</i>	84	24	8
<i>Physa acuta</i>	70	43	0
<i>Gyraulus chinensis</i>	40	36	0

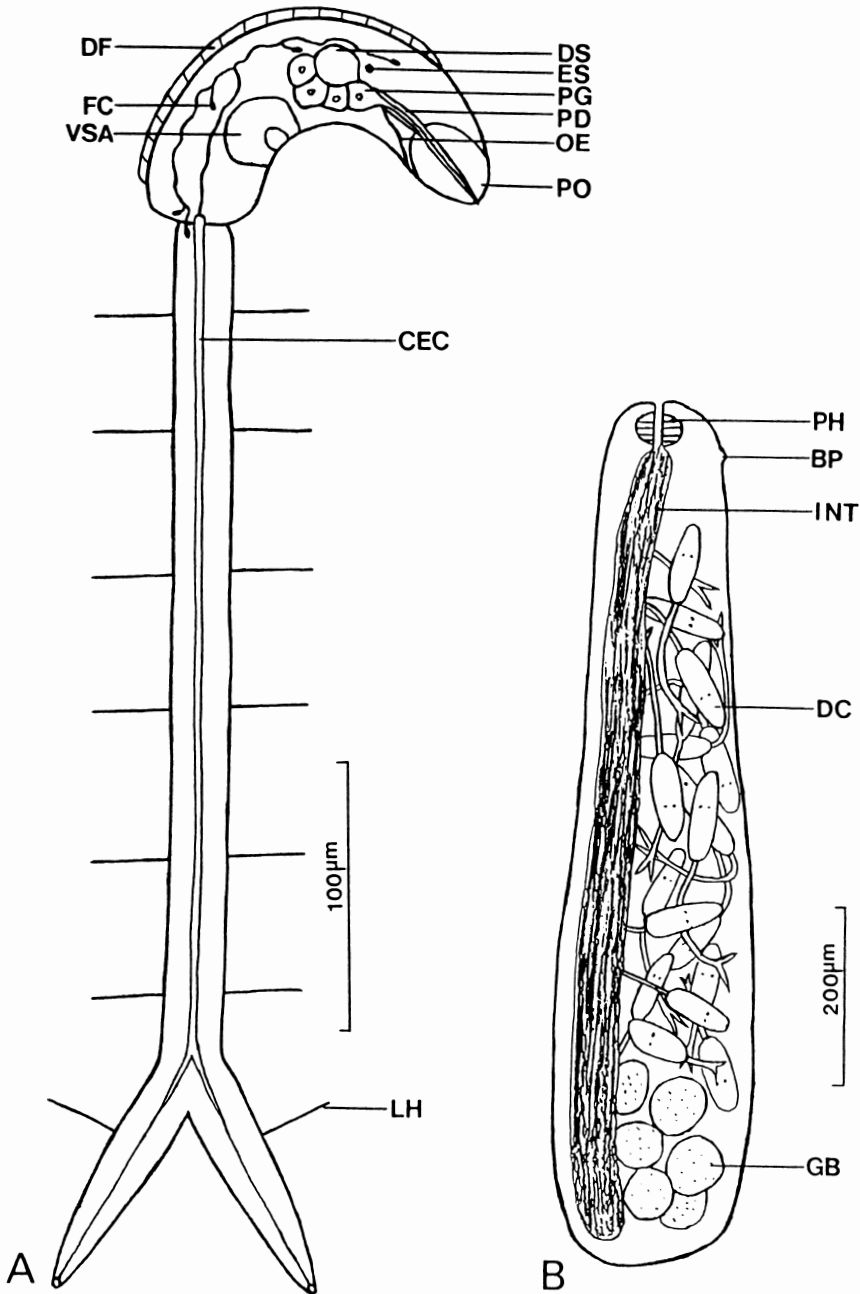


Fig. 1 *Clinostomum complanatum* A; Entire cercaria. B; Redia with germ balls and cercariae.

Abbreviations

BP	Birth pore	FC	Flame cell	PG	Penetration glands
CEC	Caudal excretory canal	GB	Germ ball	PH	Pharynx
DC	Developing cercaria	INT	Intestine	PO	Penetration organ
DF	Dorsal fin	LH	Long hair	OE	Oesophagus
DS	Dilated structure	PD	Penetration ducts	VSA	Ventral sucker analogue
ES	Eye spot				

Table 2 Measurements of *Clinostomum complanatum* cercariae (in μm)

	Authors		
	Lo <i>et al.</i> (1982)	Present authors*	Present authors [†]
Total length	528	497–549 (516) [‡]	479–546 (510)
Body length	132	104–124 (114)	110–122 (116)
width	33	35– 40 (36)	32– 38 (34)
Tail stem length	195–360	294–319 (304)	277–319 (298)
width	13– 20	21– 27 (24)	22– 27 (24)
Furca length	60–100	76–107 (93)	76–107 (94)
Penetration organ length	27	35– 43 (40)	35– 44 (39)
width	20.5	22– 26 (24)	19– 25 (21)
Eye spot	5.08	4.5– 6.3 (5.3)	4.5– 6.3 (5.3)

*Measurements of mature cercariae removed from *Lymnaea japonica*

[†]Measurements of mature cercariae removed from *Lymnaea ollula*

[‡]Figures in parentheses are the means

The caudal excretory canal was connected with the excretory bladder in the anterior extremity of the tail stem and opened at the top of the furcae.

Redia contained 10–25 germ balls and cercariae in various developmental stages (Fig. 1B), and measured 378–1497 \times 146–311 (Table 3). The pharynx was located in the anterior subterminal of the body and measured 32–56 \times 37–66. The gut extended toward the posterior end of the body and measured 316–1355 in length. The birth pore was located just

beneath the pharynx.

The morphological features and measurements of the mature cercariae and rediae removed from *L. ollula* agreed well with those removed from *L. japonica* in the present study (Tables 2 and 3).

Discussion

The present study clarified the cercarial and redial forms of *C. complanatum* in Japan for the first

Table 3 Measurements of *Clinostomum complanatum* rediae (in μm)

	Authors		
	Lo <i>et al.</i> (1982)	Present authors*	Present authors [†]
Body length	1100	378–1497 (778) [‡]	384–889 (641)
width	115.56	146– 311 (204)	102–218 (161)
Pharynx length	–	32– 56 (42)	28– 60 (38)
width	–	37– 66 (46)	26– 76 (42)
Intestine	–	351–1411 (728)	184–816 (590)

*Measurements of rediae removed from *Lymnaea japonica*

[†]Measurements of rediae removed from *Lymnaea ollula*

[‡]Figures in parentheses are the means

time. The morphological features and measurements of the cercariae almost corresponded with the descriptions by Lo *et al.* (1982). However, Lo *et al.* (1982) did not mention the existence of long hairs on the tail stem and furcae. Long hairs on the tail stem and furcae of the cercariae were recognized in the present study as well as in *Clinostomum marginatum* by Krull (1934), while *Clinostomum giganticum* cercariae were devoid of these structures (Agarwal, 1959). The presence of long hairs on the tail stem and furcae of the cercariae is one of the key characteristics in the classification of *Clinostomum*.

Agarwal (1959) divided *Clinostomum* cercariae into three groups on the basis of the structure of the digestive tract. Olsen (1974) and Yamaguti (1975) described that *C. complanatum* cercariae belonged to the pharyngeal group. However, their descriptions were quoted from a paper (Krull, 1934) on *C. marginatum* cercariae which were considered to be synonymous with *C. complanatum*. *C. marginatum* cercariae had a large delicate pharynx. On the other hand, the digestive tract of the present cercaria was morphologically simpler than that of *C. marginatum*. The mouth led directly to esophagus and the pharyngeal structure was absent. Accordingly, *C. complanatum* cercariae should be regarded as a member of the apharyngeal group.

Although several species of fish and bird hosts of *C. complanatum* have been reported in the eastern part of Tottori Prefecture (Aohagi *et al.*, 1992a, b, 1993), no reports on the snail host of this fluke have been made so far. The present study used the freshwater snails which were common in ditches and rice fields of this area, and experimentally clarified that *C. complanatum* larvae could be produced in both *L. japonica* and *L. ollula*. Therefore, it is likely that both *L. japonica* and *L. ollula* serve as snail hosts of this fluke in the eastern part of Tottori Prefecture.

As to the snail host of *C. complanatum*, 3 species of snails (*Lymnaea auricularia swinhoi*, *Lymnaea stagnalis* and *Radix ovata*) have been reported in USSR and Taiwan (Grabda-Kazubaska, 1974; Lo *et al.*, 1982). Both *L. japonica* and *L. ollula* were newly recorded here as snail hosts of *C. complanatum*.

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