

***Clinostomum complanatum* Infection in *Carassius* spp. Collected from Some Ponds and Rivers in Tottori and Shimane Prefectures, Japan**

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Abstract

During the period May 1990 through October 1993, a total of 549 *Carassius* spp. were collected from 9 localities in Tottori and Shimane Prefectures in order to clarify their *Clinostomum* infection in this district. A total of 1,197 *Clinostomum* metacercariae were found in 182 *Carassius* spp. collected from all the localities. Each infected fish harbored 1 to 74 metacercariae. The main habitat of *Clinostomum* metacercariae in the fishes was determined to be the muscles and the tissues around the gills. Some of excysted metacercariae were inoculated around the eye orbits of 27 hens. Thirty-four adult flukes were recovered from 17 hens on the 6th day after inoculation. These flukes were identified as *Clinostomum complanatum* (Rudolphi, 1814) on the basis of the morphological features and measurements of the metacercariae, adult flukes and eggs. The data from the present study indicate that *Carassius* spp. infected with *C. complanatum* is widely distributed in both Tottori and Shimane Prefectures.

Key words: *Carassius* spp., *Clinostomum complanatum*, metacercaria, Tottori and Shimane Prefectures

Introduction

Twelve human cases of *Clinostomum* infection have been reported in Japan (Yamashita, 1938; Hori, 1942; Kamo *et al.*, 1962; Sakaguchi *et al.*, 1966; Sano *et al.*, 1980; Kumada *et al.*, 1983; Hirai *et al.*, 1987; Furukawa and Miyazato, 1987; Yamane *et al.*, 1989; Umezaki *et al.*, 1990; Yoshimura *et al.*, 1991; Isobe *et al.*, 1993). Among them, the flukes detected in 5 human cases were identified as *Clinostomum complanatum* (Rudolphi, 1814). These human cases occurred after having eaten raw freshwater fish. Therefore, it is likely that freshwater fish is the source of the human infection with *C. complanatum*.

Three species of *Carassius* (*C. carassius* = *C. carassius buergeri*, *C. gibelio langsdorfi* and *C. cuvieri*) have been recorded as the second intermediate hosts of *C. complanatum* in Japan (Yamaguti, 1933; Aohagi *et al.*, 1992a). Aohagi *et al.* (1993a)

revealed that *C. cuvieri* which was purchased from fish dealers in Tottori City harbored lots of *C. complanatum* metacercariae. These fishes were collected from local ponds and lakes. *Carassius* spp. is widely distributed in the rivers, ponds and lakes in both Tottori and Shimane Prefectures. The present study was carried out in order to clarify *C. complanatum* infection in *Carassius* spp. inhabiting in this district.

Materials and Methods

Fish:

During the period May 1990 through October 1993, a total of 549 *Carassius* spp. (3.8–26.7cm in body length) were collected from 9 localities in Tottori and Shimane Prefectures (Fig. 1). These localities consisted of 3 ponds (Koyamacho-minami [1], Nokyo [2] and Miuchidani [3]) and 5 rivers (Nishikurayoshimachi [4], Abe [5], Yasugicho [6], Maehashima [7], Nokatacho [8] and Nakamizo [9]). Maehashima [7] and Nokatacho [8] located the lower and upper reaches of the same river, respectively.

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青萩芳幸 柴原壽行 (鳥取大学医学部附属動物実験施設)

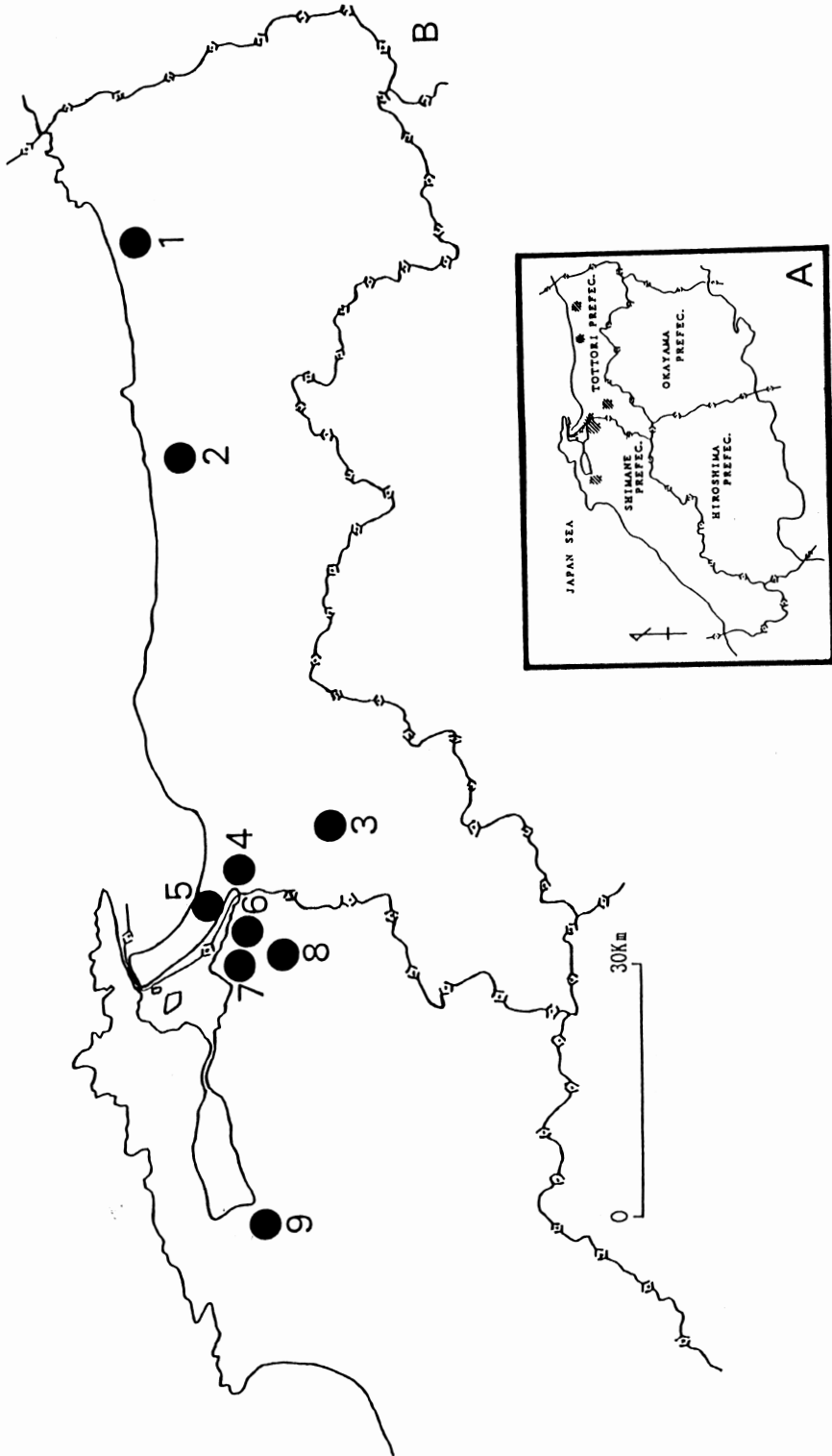


Fig. 1 A) Map showing the surveyed areas (shaded portions). B) A map showing 9 localities in Tottori and Shimane Prefectures where *Carassius* sp. was collected.

Detection of parasite:

Detection of *Clinostomum* metacercariae from fish was done by the method previously described (Aohagi *et al.*, 1993a). After measuring the encysted metacercariae under the dissecting microscope, these metacercariae were excysted by exposing them in artificial gastric juice (distilled water 1,000ml, pepsin 1g, 36% HCl 7ml; pH 1.3).

Experimental infection to animals:

Twenty-seven hens (White Leghorn, 16–24wks old, weighing 0.96–1.38kg) purchased from the local poultry farm were used. The inoculation of the metacercariae were made according to the method described by Ismail and Issa (1987). Namely, 10 excysted metacercariae were inoculated around the orbits of both eyes of each hen using a Pasteur pipette. These hens were given the mixed feed of grains. Then they were sacrificed on the 6th day after inoculation. The eye orbits, nasolacrimal canals and complete digestive tract of hens were macroscopically examined for *Clinostomum* infection. The flukes were picked up from the mucous membrane of hens using a forceps.

Flukes and eggs:

Adult flukes were put in 0.85% saline solution for 6 hours to let them produce eggs. The eggs were preserved in 10% formalin. Metacercariae and adult flukes were fixed in 70% alcohol under the pressure of a cover glass, stained with Borax-carmin, cleared in xylene and mounted in Canada-balsam. These specimens were used for morphological observations and measurements.

Results

Prevalence and intensity of the metacercarial infection of Clinostomum in fish:

As shown in Table 1, *Clinostomum* metacercariae were detected from *Carassius* spp. collected from all the localities. The prevalence rates of *Clinostomum* metacercariae in each locality ranged from 5.0% (Nakamizo [9]) to 52.5% (Nishikurayoshimachi [4]). The average number of metacercariae per positive fish in each locality ranged from 1.0 to 9.8. The maximum number of metacercariae in a positive fish was 74 in Nishikurayoshimachi [4].

Recovery site of metacercariae:

A total of 1,197 metacercariae found in 182 *Carassius* spp. involved 645 originated from the body muscles, 124 from the muscles at the pectoral fins, 45 from the muscles at the abdominal fins, 106 from the cheek muscles, 256 from the tissues around the gills, 10 from the gills and 11 from the pharynx.

Morphology of metacercariae:

Metacercariae obtained from each locality were morphologically similar to each other. The cysts were yellowish in color, spherical or elliptical in shape and measured 1.57–2.66mm in diameter (Fig. 2). Excysted metacercariae were linguiform in shape with a slight contraction at the level of the ventral sucker, measured 3.58–6.99mm in length and 1.09–2.16mm in width (Fig. 3). The body surface was covered with minute single spines. The oral sucker was located in the anterior subterminal of the body, elliptical in shape, measured 0.176–0.420mm in length and 0.252–0.509mm in width. The ventral sucker was located in the anterior third of the body, almost spherical in shape, larger than the oral sucker and measured 0.630–1.046mm in diameter. Reproductive organs were immature and situated behind the ventral sucker.

Recovery of adult flukes:

In the experimental infection of hens, a total of 34 flukes were recovered from the surface of the mucous membrane of the upper and lower eyelids, nasolacrimal canals and pharynx of 17 out of 27 hens which have been inoculated with the metacercariae. The number of flukes per infected hen was 1 to 5. All the flukes were sexually in maturity, and had eggs in their uteri and uterine sacs (Fig. 4).

Morphology of adult flukes and their eggs:

The adult flukes were elliptically elongated in shape, measured 4.62–6.52mm in length and 1.87–2.78mm in width. The body surface was aspinous. The oral sucker was elliptical in shape, measured 0.303–0.379mm in length and 0.339–0.481mm in width. The ventral sucker was almost spherical in shape and measured 0.734–0.968mm in diameter. Reproductive organs were more developed than those of metacercariae.

Table 1 Prevalence and intensity of *Clinostomum complanatum* metacercariae (Mc) in *Carassius* spp. collected from 9 localities in Tottori and Shimane Prefectures

Locality (Pond or river name)	[No.]*	Date of survey	No. of fish		Prevalence rate (%)	Average No. of Mc in a positive fish (Min.-Max.)	
			examined	infected			
Tottori City Koyamacho-minami (Koyama Pond)	[1]	October ~July	1991 1992	130	25	19.2	4.2 (1-24)
Tougo-cho Nokyo (Tougo Pond)	[2]	May	1990	59	12	20.3	1.3 (1- 3)
Aimi-cho Miuchidani (Nakazodani Pond)	[3]	January	1993	37	5	13.5	1.0
Yonago City Nishikurayoshimachi (Kamo River)	[4]	March ~October	1993 1993	118	62	52.5	9.8 (1-74)
Yonago City Abe (Yone River)	[5]	October ~December	1992 1992	70	33	47.1	7.2 (1-67)
Yasugi City Yasugicho (Kido River)	[6]	February	1993	63	27	42.9	4.4 (1-39)
Yasugi City Maehashima (Yoshida River)	[7]	February ~October	1993 1993	39	13	33.3	7.9 (1-46)
Yasugi City Nokatacho (Yoshida River)	[8]	February	1993	13	4	30.8	1.0
Hikawa-cho Nakamizo (Shintate River)	[9]	May	1990	20	1	5.0	1.0
Total				549	182	33.2	6.6

*The Nos. are indicated in Fig. 1.

Eggs were elliptical in shape, unembryonated, measured 0.119–0.136mm in length and 0.067–0.074mm in width.

The morphological features and measurements of the metacercariae, adult flukes and eggs corresponded with the descriptions on *C. complanatum* by Kagei *et al.* (1984, 1988) and Aohagi *et al.* (1993a, b). Therefore, these flukes were identified as *C. complanatum*.

Discussion

The present authors have been investigating the natural hosts of *C. complanatum* in the eastern part of Tottori Prefecture (Aohagi *et al.*, 1992a, b, 1993b).

As a result, it was found that 5 species of herons and egrets served as definitive hosts of *C. complanatum* and 6 species of freshwater fish as the second intermediate hosts, respectively. In addition, it was experimentally clarified that both *Lymnaea japonica* and *Lymnaea ollula* inhabiting in this district served as the first intermediate hosts of *C. complanatum* (Aohagi *et al.*, 1993c). The life cycle of this fluke may have been maintaining among these creatures in this district.

It was clarified that two species of *Carassius* (*C. gibelio langsdorfi* and *C. cuvieri*) inhabited in Koyama Pond (Nomura, 1993). One hundred and twenty-nine out of 130 *Carassius* spp. collected from Koyama Pond (Koyamacho-minami [1]) in

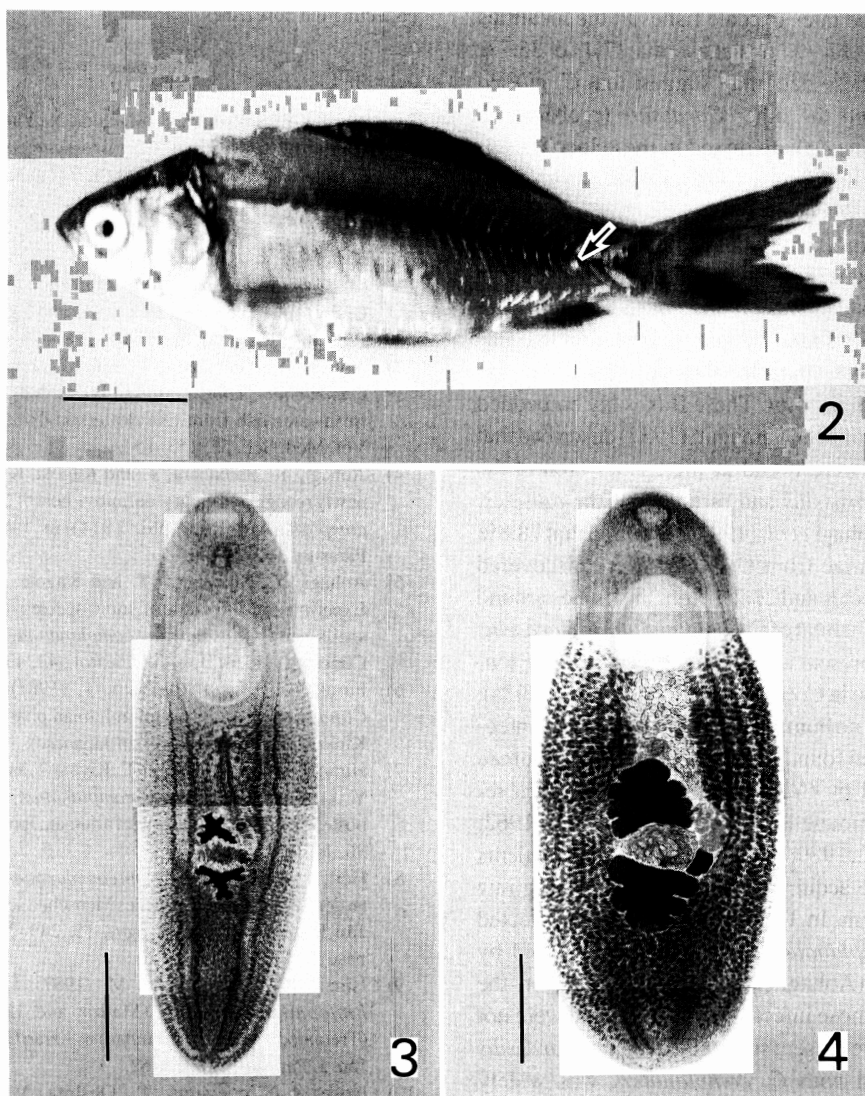


Fig. 2 A skinned second intermediate host, *Carassius* sp. An arrow shows the metacercaria of *Clinostomum complanatum* which lies embedded in the body muscles. Bar: 10mm.

Fig. 3 An excysted metacercaria of *Clinostomum complanatum* (mounted specimen). Bar: 1mm.

Fig. 4 An adult fluke of *Clinostomum complanatum* recovered from the mucous membrane of the eyelid of an experimentally inoculated hen (mounted specimen). Bar: 1mm.

the present study were identified as *C. gibelio langsdorfi* and one *C. cuvieri*. Twenty-five out of 129 *C. gibelio langsdorfi* were infected with *C. complanatum* metacercariae. On the other hand, the morphological features of *Carassius* spp. collected from all the localities except Koyamacho-minami

[1] resembled those of *C. gibelio langsdorfi* from Koyamacho-minami [1]. *C. gibelio langsdorfi* are classified into two types (triploid and tetraploid) based on their karyotypic analysis (Kobayashi, 1971). This species propagates unisexually by gynogenesis and generally consists of female fishes. However,

the prevalence rates of male fishes in the localities ranged from 17.9% (Maehashima [7]) to 35.7% (Abe [5]). These data may suggest that *C. gibelio langsdorfi* and the other *Carassius* (probably *C. carassius buergeri*) are mixed in these localities. It is not easy to distinguish *C. gibelio langsdorfi* from *C. carassius buergeri* by the morphological features. Therefore, the species names of the host fishes were not specified in the present study.

The encysted metacercariae of *C. complanatum* could be easily found by visual inspection because they were conspicuous for about 1.6–2.6mm in size and yellowish in color. These flukes lay embedded in the tissues of fish. Yamaguti (1933) described that these flukes were found in the tissues around the gills of *C. carassius* and rarely from the muscles. However, Aohagi *et al.* (1993a) reported that 78.8% of metacercariae from *C. cuvieri* were discovered from the muscles and 21.2% from the tissues around the gills. The habitat of *C. complanatum* in *Carassius* spp. in the present study showed the similar tendency to those in *C. cuvieri* by Aohagi *et al.* (1993a).

Although no human cases of *Clinostomum* infection have been found in Tottori Prefecture, 3 human cases caused by *Clinostomum* spp. have been reported in Shimane Prefecture (Kamo *et al.*, 1962; Yamane *et al.*, 1989; Isobe *et al.*, 1993). The patients seem to have acquired the infection by eating raw freshwater fish. In Tottori City, *C. cuvieri* infected with *C. complanatum* metacercariae was sold by fish dealers (Aohagi *et al.*, 1993a). However, the localities of those infected fishes collected were not specified. The present study clarified that *Carassius* spp. infected with *C. complanatum* was widely distributed in both Tottori and Shimane Prefectures. Among these fish, *Carassius* spp. collected from Koyamacho-minami [1], Nokyo [2], Maehashima [7] and Nakamizo [9] was sold by fish dealers. Therefore, it is needed to pay special attention to eating raw freshwater fishes which were collected from the local rivers and ponds as there is a risk of the human infection with *C. complanatum* caused by these sources.

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References

- 1) Aohagi, Y., Shibahara, T., Machida, N., Yamaga, Y. and Kagota, K. (1992a): *Clinostomum complanatum* (Trematoda: Clinostomatidae) in five new fish hosts in Japan. *J. Wildl. Dis.*, 28, 467–469.
- 2) Aohagi, Y., Shibahara, T., Machida, N., Yamaga, Y., Kagota, K. and Hayashi, T. (1992b): Natural infections of *Clinostomum complanatum* (Trematoda: Clinostomatidae) in wild herons and egrets, Tottori Prefecture, Japan. *J. Wildl. Dis.*, 28, 470–471.
- 3) Aohagi, Y., Shibahara, T. and Kagota, K. (1993a): *Clinostomum complanatum* (Trematoda) infection in freshwater fish from fish dealers in Tottori, Japan. *J. Vet. Med. Sci.*, 55, 153–154.
- 4) Aohagi, Y., Shibahara, T. and Kagota, K. (1993b): A newly recognized natural definitive host of *Clinostomum complanatum* (Rudolphi, 1814) in Japan. *Jpn. J. Parasitol.*, 42, 44–46.
- 5) Aohagi, Y., Shibahara, T. and Kagota, K. (1993c): Experimental infection of some species of freshwater snails with *Clinostomum complanatum* (Trematoda: Clinostomatidae). *Jpn. J. Parasitol.*, 42, 493–498.
- 6) Furukawa, T. and Miyazato, T. (1987): A case of *Clinostomum* sp. infection in human pharynx. *Med. J. Kinki Univ.*, 12, 665–669 (in Japanese).
- 7) Hirai, H., Ooiso, H., Kifune, T., Kiyota, T. and Sakaguchi, Y. (1987): *Clinostomum complanatum* infection in posterior wall of the pharynx of a human. *Jpn. J. Parasitol.*, 36, 142–144.
- 8) Hori, K. (1942): *Clinostomum complanatum* (Rud. 1819) an der Rachenschleimhaut des Menschen schmarotzend. *Jibi Inkoka* (Otolaryngology), 15, 249–252 (in Japanese).
- 9) Ismail, N. S. and Issa, I. M. (1987): Life cycle of *Philophthalmus gralli* (Mathis and Leger, 1910) (Trematoda: Philophthalmidae) in Azraq Oasis, Jordan. *Jpn. J. Parasitol.*, 36, 53–62.
- 10) Isobe, A., Yoneyama, T., Ohshita, M., Hojo, N., Fukushima, T., Shiwaku, K., Yamane, Y. and Kinoshita, S. (1993): The 12th human case of *Clinostomum* sp. infection in Japan. *Jpn. J. Parasitol.*, 42, (Suppl.), 165 (in Japanese).
- 11) Kagei, N., Yanohara, Y., Uchikawa, R. and Sato, A. (1984): On the yellow grubs, metacercariae of *Clinostomum complanatum* (Rudolphi, 1819), found in the cultured loach. *Jpn. J. Parasitol.*, 33, 59–62.
- 12) Kagei, N., Yanohara, Y., Uchikawa, R. and Sato, A. (1988): Natural infection with *Clinostomum complanatum* (Rud., 1819) in the birds of southern Japan. *Jpn. J. Parasitol.*, 37, 254–257.
- 13) Kamo, H., Ogino, K. and Hatsushika, R. (1962): A unique infection of man with *Clinostomum* sp., a small trematode causing acute laryngitis. *Yonago Acta Med.*, 6, 37–40.
- 14) Kobayashi, H. (1971): A cytological study on

- gynogenesis of the triploid ginbuna (*Carassius auratus langsdorfi*). Zool. Mag., 80, 316–322 (in Japanese).
- 15) Kumada, N., Mizuno, S., Kawamoto, F., Fujioka, H. and Nakanishi, K. (1983): A case of *Clinostomum* sp. infection in Aichi Pref., Japan. Jpn. J. Parasitol., 32, (Suppl.), 17 (in Japanese).
 - 16) Nomura, Y. (1993): Notes on some fishes in Lake Koyama, Tottori City. Bull. Tottori Prefectural Museum, 30, 1–10 (in Japanese).
 - 17) Sakaguchi, Y., Yamamoto, T. and Yamada, N. (1966): *Clinostomum* sp. a trematoda parasite, removed from larynx of a man. End. Dis. Bull. Nagasaki Univ., 8, 40–44 (in Japanese).
 - 18) Sano, M., Mogi, K. and Kamegai, S. (1980): A case of *Clinostomum* sp. throat infection. Jibi Inkoka (Otolaryngology), 52, 1037–1039 (in Japanese).
 - 19) Umezaki, T., Shin, T., Oda, M., Kifune, T. and Mogi, M. (1990): A case of acute laryngitis caused by *Clinostomum complanatum* with a complaint of throat irritation. Jibi to Rinsho (Otologia), 36, 665–668 (in Japanese).
 - 20) Yamaguti, S. (1933): Studies on the helminth fauna of Japan. Part 1. Trematodes of birds, reptiles and mammals. Jpn. J. Zool., 5, 66–71.
 - 21) Yamane, Y., Osaki, Y., Fujino, N. and Nagasako, R. (1989): The 8th human case of *Clinostomum* sp. infection in Japan. Jpn. J. Parasitol., 39, (Suppl.), 117 (in Japanese).
 - 22) Yamashita, J. (1938): *Clinostomum complanatum*, a trematode parasite new to man. Annot. Zool. Japon., 17, 563–566.
 - 23) Yoshimura, K., Ishigooka, S., Satoh, I. and Kamegai, S. (1991): *Clinostomum complanatum* from the pharynx of a woman in Akita, Japan. A case report. Jpn. J. Parasitol., 40, 99–101.