

## Demonstration of the second intermediate hosts of *Clinostomum complanatum* in Korea

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**Abstract:** A species of metacercariae recovered from the fresh-water fish, collected from Kaumji (Pond), Kaechonji (Pond) and Ssanggyechon (River), Uisong-gun, Kyongsangbuk-do, Korea, was identified as *Clinostomum complanatum* by morphological observation and experimental infection to chicks. The excysted metacercariae, tongue-shaped and progenetic, were 3.28-4.27 mm in length and 0.94-1.46 mm in width. The adult flukes recovered from the chicks four days after infection were 4.20-4.86 mm long and 1.14-1.49 mm wide. Twelve species of the fresh-water fish were found to be infected with the metacercariae. The infection rate ranged from 1.6% (*Zacco temminckii*) to 88.9% (*Acheilognathus rhombea* and *Microphysogobio yaluensis*). The intensity was highest in *Carassius auratus* (13.0/fish infected) and the abundance (relative density) was highest in *A. rhombea* (7.8/fish examined). This survey demonstrated for the first time the source of human infection by *C. complanatum* in Korea.

**Key words:** *Clinostomum complanatum*, metacercariae, second intermediate hosts, fresh-water fish, Uisong-gun, Korea

### INTRODUCTION

*Clinostomum complanatum* (Rudolphi, 1814) is a digenetic trematode which naturally parasitizes the throat and esophagus of piscivorous birds (Yamaguti, 1958). Sometimes, the fluke causes pharyngitis in human beings. Human infection is known to result from eating raw fresh-water fish. Many species of fresh-water fish were recorded as the second intermediate hosts of *C. complanatum* (Yamaguti, 1933; Aohagi *et al.*, 1992a & 1993).

In Korea, Chung *et al.* (1995) reported the first human case of *Clinostomum* infection. It is suggested that the natural life cycle of *C. complanatum* is maintained in Korea. However,

to the best of our knowledge, no report exists on the sources of human infection, i.e., the second intermediate hosts of *C. complanatum*, and on the morphology of the metacercaria in Korea. Authors morphologically identified a species of metacercariae from 12 fresh-water fish as *C. complanatum* and confirmed it by obtaining adult worms from the chicks experimentally infected with the metacercariae. In this report, authors present the morphological observation and measurements of the metacercariae, the list of the second intermediate hosts, and the infection status of the hosts with the metacercariae of *C. complanatum*.

### MATERIALS AND METHODS

A total of 741 fresh-water fish were collected from Kaumji (Pond), Kaechonji (Pond) and Ssanggyechon (River), located in Uisong-gun, Kyongsangbuk-do, Korea (Fig. 1) from April to

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August, 1994. The fish collected were eviscerated to prevent autodigestion and transported to our laboratory.

The species of the fish was identified according to the keys described by Kim and Kang (1993). The weight and length of each fish were measured after species identification. Whole body was examined macroscopically for the presence of the metacercaria of *Clinostomum complanatum* and the metacercaria, if any, was confirmed under a dissecting microscope. In order to collect the metacercariae, artificial digestion technique was employed. The flesh was taken from the fish and mixed with artificial gastric juice. The juice consisted of 3.0 ml of diluted hydrochloric acid and 0.3 gm of pepsin per 100 ml of distilled water. Beakers containing the mixture were incubated in a water-bath at 37°C for 30 minutes. The mixture was stirred with a magnetic bar and allowed to stand for a minute to gather the isolated larvae on the central portion of the beaker. Ten excysted metacercariae were inoculated to each chick according to the methods described by Aohagi and Shibahara (1994). The chicks were sacrificed four days after the experimental infection and adult flukes were recovered from the pharynx of the chicks.

Some of the metacercariae and adult flukes were fixed between 2 slide glasses with 70% ethyl alcohol or 10% formalin and stained with acetocarmine. The stained specimens were

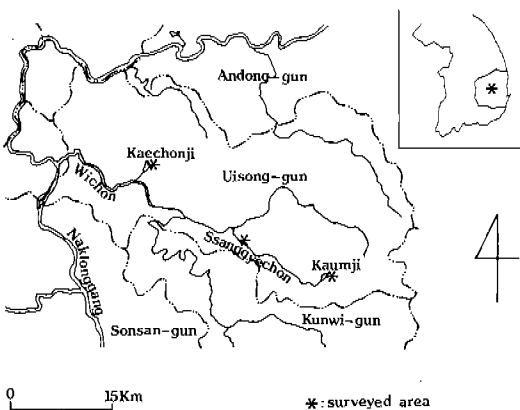
used for morphological observations and measurements.

The terms used for explanation of the infection status of the fresh-water fish abided by the recommendations of an Ad Hoc committee of the American Society of Parasitologists (Margolis *et al.*, 1982). The term intensity refers to number of individuals of a particular parasite species in each infected host. The term abundance or relative density refers to mean number of individuals of a particular parasite species per host examined i.e., mean intensity  $\times$  prevalence/100.

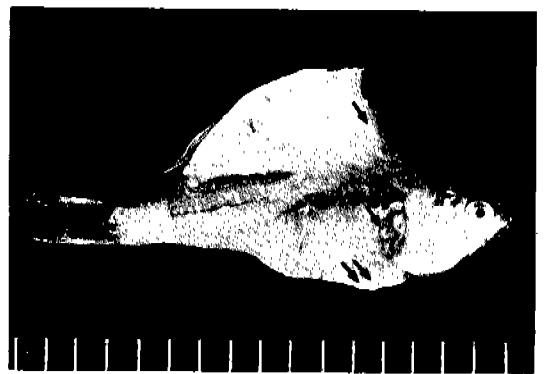
## RESULTS

### 1. Morphology of metacercariae of *Clinostomum complanatum*

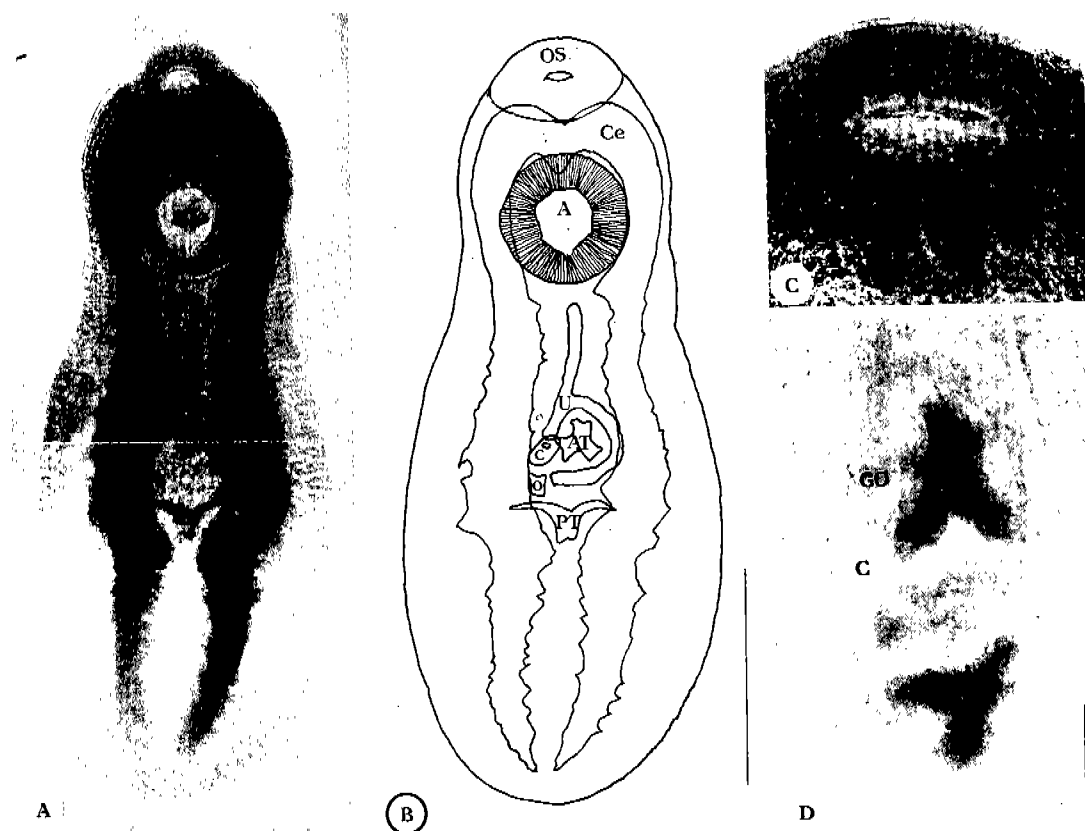
The metacercariae were recovered mainly from muscles, tissues around gills, and fins of the fish (Fig. 2). The excysted metacercariae were tongue-shaped, slightly attenuated at the postacetabular level and measured 3.28-4.27 mm in length by 0.94-1.46 mm in width (Fig. 3A & B). No tegumental spines were observed on the body surface. The oral sucker, anteriorly subterminal, measured 0.22-0.32 mm long and 0.27-0.43 mm wide. The ventral sucker was located in the anterior third of the body and measured 0.51-0.77mm long and 0.52-0.75 mm wide. The pharyngeal bulb was oval and well-developed (Fig. 3c). The intestine, bifurcated and voluminous, possessed outpocketings in the middle and posterior portions behind the acetabulum. The genital



**Fig. 1.** The map showing the surveyed areas of Kaumji (Pond), Kaechonji (Pond) and Ssanggyechon (River). Uisong-gun, Kyungsangbuk-do, Korea.



**Fig. 2.** A crucian carp, *Carassius auratus* infected with many metacercariae (arrows) of *Clinostomum complanatum*.



**Fig. 3.** **A.** An acetocarmine stained metacercaria of *C. complanatum*. Bar indicates 1 mm. **B.** Drawing of A (A, acetabulum; AT, anterior testis; C, cirrus; Ce, ceca; O, ovary; PT, posterior testis; U, uterus). **C.** Pharyngeal bulb. Bar indicates 0.1 mm. **D.** Genital opening located at the right side of the anterior testis. Bar indicates 0.2 mm.

**Table 1.** Comparative measurements of the excysted metacercariae of *Clinostomum complanatum* from different fresh-water fish

		present specimens (1994)	Kagei <i>et al.</i> (1984)	Aohagi & Shibahara (1994)
No. exam.		25	10	n.d. <sup>a)</sup>
Body	L <sup>b)</sup>	3.28-4.27 mm	4.64-8.24	3.58-6.99
	W <sup>c)</sup>	0.94-1.46	1.39-2.09	1.09-2.16
Oral sucker	L	0.22-0.32	0.30-0.68	0.18-0.42
	W	0.27-0.43	0.28-0.49	0.25-0.51
Acetabulum	L	0.51-0.77	0.73-1.03	0.63-1.05
	W	0.52-0.75	0.89-1.15	—

<sup>a)</sup>n.d. means not described. <sup>b)</sup>L means length. <sup>c)</sup>W means width.

primordia were situated in the middle third of the body. The testes were tandem and deeply lobed. The uterus extended from the intertesticular to postacetabular region. The ovary was small and indistinct between the two

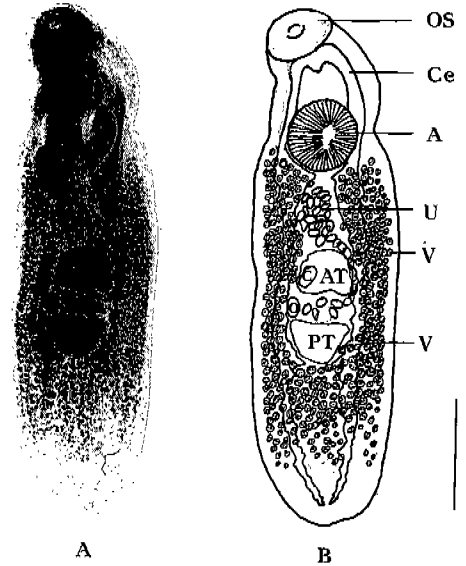
testes. The genital pore opened at the right side of the anterior testis (Fig. 3D). The comparative measurements of the metacercariae are given in Table 1.

## 2. Morphology of adults of *C. complanatum*

The adult worms obtained from chicks four days after experimental infection appeared linguiform and were 4.20-4.86 mm long by 1.14-1.49 wide (Fig 4A & B). Body width abruptly expanded from the level of the center of the ventral sucker. Posterior extremity more rounded than the anterior. No spine distinguished on the tegument. Oral sucker subterminal and somewhat transverse, measured 0.28-0.34 mm long by 0.37-0.44 mm wide. Pharynx and esophagus obscure. Ceca bifurcated immediately behind the oral sucker and contained materials stained in brown color. Ventral sucker, 0.60-0.72 mm long and 0.64-0.72 mm wide, located in the anterior one third. The orifice of the ventral sucker longitudinally elongated. Testes separated by the uterus. Anterior testis measured 0.38-0.44 mm by 0.41-0.62 mm and posterior one, 0.31-0.40 mm by 0.53-0.71 mm. Ovary oval, 0.21-0.29 mm by 0.14-0.21 mm, and placed between testes, touching the right ceca. Cirrus pouch unclear. Uterus situated between the ventral sucker and the posterior testis. Vitellaria follicular, and distributed from the postacetabular level to the caudal end of the body along the lateral margins. Eggs in uterus measured 113-149  $\mu\text{m}$  long by 74-88  $\mu\text{m}$  wide and possessed an operculum, 21-27  $\mu\text{m}$  wide. The comparative measurements of *C. complanatum* adult worm are shown in Table 2.

## 3. Infection status of fresh-water fish with *C. complanatum* metacercariae

Table 3 shows the prevalence, intensity, and abundance (relative density) of infection with *C. complanatum* metacercariae in fresh-water fish. Among sixteen species of fish collected, 12 species, the oily bitterling, *Acheilognathus koreensis*, the flat bitterling, *A. rhombea*, the striped bitterling, *A. yamatsutae*, the crucian carp, *Carassius auratus*, the spined loach, *Cobitis sinensis*, *Microphysogobio yaluensis*, the southern top-mouthed minnow, *Pseudorasbora parva*, the black striped gudgeon, *Pungtungia herzi*, the bride bitterling, *Rhodeus uyekii*, the korean gudgeon,



**Fig. 4.** An acetocarmine stained adult worm of *C. complanatum* recovered from the pharynx of an experimentally infected chick. **A.** Photomicrograph. **B.** Drawing of A (A, acetabulum; AT, anterior testis; C, cirrus; Ce, ceca; O, ovary; PT, posterior testis; U, uterus; V, vitellaria). Bar indicates 1 mm.

*Squalidus chankaensis tsuchigae*, the korean slender gudgeon, *S. gracilis majtmae* and the dark chub, *Zacco temminckii* were found to be infected with *C. complanatum* metacercariae. The infection rate ranged from 88.9 (A. *rhombea* and *M. yaluensis*) to 1.6% (*Z. temminckii*).

In the intensity of infection with *C. complanatum* metacercaria, *C. auratus* was the most heavily infected and the average number of the metacercariae per infected fish was 13.0, followed by *A. koreensis* 9.0, *A. rhombea* 8.8 and *P. herzi* 8.7. The relative density (abundance) of *C. complanatum* metacercaria was the highest in *A. rhombea*, 7.8, followed by *M. yaluensis* 4.8, and *A. koreensis* and *P. herzi*, 4.3 of each.

## DISCUSSION

From this study, it was confirmed that *Clinostomum complanatum* has been indigenously distributed in Korea. The

**Table 2.** Comparison of the measurements of *C. complanatum* adult worms from chicks, a human and herons

No. exam.		Present specimens (1995)	Chung <i>et al.</i> (1995)	Kagei <i>et al.</i> (1988)
		5	1	10
Body	L	4.20-4.86 mm	4.74	3.21-5.58
	W	1.14-1.49	1.05	1.94-2.36
Oral sucker	L	0.28-0.34	0.29	0.24-0.40
	W	0.37-0.44	0.43	0.34-0.46
Pharynx	L	—	—	0.12-0.16
	W	—	—	0.11-0.21
Acetabulum	L	0.60-0.72	0.72	0.45-0.83
	W	0.64-0.72	0.64	0.61-0.80
Testes Anterior	L	0.38-0.44	0.53	0.31-0.76
	W	0.41-0.62	0.51	0.61-1.04
Posterior	L	0.31-0.40	0.35	0.20-0.44
	W	0.53-0.71	0.55	0.26-1.21
Ovary	L	0.21-0.29	0.15	0.19-0.38
	W	0.14-0.21	0.12	0.21-0.87
Cirrus pouch	L	—	0.29	0.28
	W	—	0.16	0.18
Eggs	L	113-149 $\mu$ m	101-123	110-124
	W	74-88	63-83	62-72
	Op <sup>a)</sup>	21-27	22-27	20-26
Host		Chick	Human	Heron

<sup>a)</sup>Op means width of operculum.

morphological features and measurements corresponded well with the previous descriptions on *C. complanatum* by Kagei *et al.* (1984), Aohagi and Shibahara (1994), Kagei *et al.* (1988) and Chung *et al.* (1995). Concerning the presence of tegumental spines on the metacercaria, there has been discrepancy among the workers. Aohagi and Shibahara (1994) reported minute single spines covering the body surface of the metacercaria, but no distinct spines were observed in the present study using a light microscope. Further study using a scanning electron microscope should be performed to confirm the presence or absence of tegumental spines.

This study revealed that twelve species of fresh-water fishes play roles as the second intermediate hosts of *C. complanatum* in Korea. Among twelve species, three species and two subspecies, *Acheilognathus koreensis*, *Microphysogobio yaluensis*, *Rhodeus uyekii*, *Squalidus chankaensis tsuchigae*, *S. gracilis majimae* are regarded as the fishes indigenous

to Korea (Kim and Kang, 1993). Therefore, these fishes might be recorded as the new second intermediate hosts of *C. complanatum*.

The fresh-water fish heavily infected by *C. complanatum* metacercaria were too severely damaged to thrive for a long time. This finding was also reported in the cultured loaches by Kagei *et al.* (1984). The metacercaria, also known as the yellow grub, caused considerable damage to the tissue of the fish hosts (Kalantan *et al.*, 1987). The carp and crucian carp are cultivated in ponds used as piscinae and sold as food in Korea. Kaumji (Pond) had also been used as a piscinae for the carp and Israeli carp (*Cyprinus carpio nudus*). Nowadays, however, it is no longer used. The infection of the fish by the yellow grub should be seriously considered because it can cause an economic loss and a public health problem.

Further study is needed to demonstrate the metacercaria from fresh-water fish indigenous to Korea because the results, if any, will be the first records of the fish as a second

**Table 3.** Infection status with *C. complanatum* metacercariae in fresh-water fishes collected from Kaumji (Pond), Kaecheonji (Pond) and Ssanggyecheon (River), Uisong-gun, Kyongsangbuk-do, Korea

Species of fish		Number of fish		Intensity		Abundance
Scientific name	Common name	Examined	Infected (%)	Mean	Range	
<i>Acheilognathus koreensis</i>	oily bitterling	40	19 (47.5)	9.0	1-37	4.3
<i>Acheilognathus rhombea</i>	flat bitterling	9	8 (88.9)	8.8	1-30	7.8
<i>Acheilognathus yamatsutae</i>	striped bitterling	48	20 (41.7)	1.8	1-6	0.7
<i>Carassius auratus</i>	crucian carp	298	77 (25.8)	13.0	1-310	3.4
<i>Cobitis sinensis</i>	spined loach	7	2 (38.6)	1.5	1-2	0.4
<i>Hemibarbus longirostris</i>	long nose barbel	3	0 (0)	0	0	0
<i>Microphysogobio yaluensis</i>		9	8 (88.9)	5.4	1-20	4.8
<i>Odontobutis platycephala</i>	korean dark sleeper	2	0 (0)	0	0	0
<i>Pseudorasbora parva</i>	southern top mouthed minnow	147	45 (32.6)	3.0	1-17	0.9
<i>Pungtungia herzi</i>	black striped gudgeon	12	6 (50.0)	8.7	1-30	4.3
<i>Rhodeus uyekii</i>	bride bitterling	12	7 (58.3)	1.4	1-3	0.8
<i>Sarcocheilichthys variegatus wakiae</i>	Oily shiner	2	0 (0)	0	0	0
<i>Squalidus chankaensis tsuchigae</i>	Korean gudgeon	4	2 (50)	1.5	1-2	0.8
<i>Squalidus gracilis majinae</i>	Korean slender gudgeon	28	4 (14.3)	2.0	1-4	0.3
<i>Zacco platypus</i>	pale chub	59	0 (0)	0	0	0
<i>Zacco temminckii</i>	dark chub	61	1 (1.6)	1	1	0.02

intermediate host of the fluke. It was unfortunate for the authors to discontinue the survey because of severe drought last year and this year in Uisong area, Kyongsangbuk-do, Korea.

The piscivorous birds, especially the heron, inhabiting in the surveyed areas are suspected as natural final hosts of *C. complanatum* in Korea, as are in Japan (Kagei *et al.*, 1984; Aohagi *et al.*, 1992b). The authors experimentally infected the chicks instead of the herons and have yet to demonstrate the natural infection in the herons. Because the wild birds are preserved by the authority, the examination of *C. complanatum* infection should be carried out after permission by the authority in the near future.

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=초록=

## 새인두흡충 제2중간숙주의 발견

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1994년 4월부터 8월까지 경북 의성군 가음지, 개천지 및 쌍계전에서 채집한 담수어에서 검출한 흡충류 피낭유충을 형태학적으로 관찰하여 새인두흡충의 피낭유충으로 동정한 후 이 피낭유충을 닭에 실험적 감염시켜 얻은 성충을 형태학적으로 관찰하여 확인하였다. 탈낭유충은 헛바닥 모양이며, 길이 3.28-4.27 mm, 폭 0.94-1.46 mm의 크기이었다. 실험감염 4일 후 닭에서 얻은 성충은 길이 4.20-4.86 mm, 폭 1.14-1.49 mm이었다. 16종의 담수어중 12종, 칼납자루, 납지리, 줄납자루, 붕어, 기름종개, 돌마자, 참붕어, 돌고기, 각시붕어, 참물개, 진물개 및 갈겨니에서 새인두흡충의 피낭유충을 검출하였다. 피낭유충의 기생율은 납지리와 돌마자에서 88.9%로 가장 높았고 개체당 감염정도는 붕어에서 13.0개로 가장 높았다. 이번 연구로 우리 나라에도 새인두흡충이 토착적으로 분포하고 있음을 알았고 담수어가 인체 감염원일 것으로 판단되었다.

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