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Clonorchis sinensis metacercarial infection in the pond smelt *Hypomesus olidus* and the minnow *Zacco platypus* collected from the Soyang and Daechung Lakes

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Abstract: The pond smelt *Hypomesus olidus* and minnow *Zacco platypus* were collected from the Soyang and Daechung Lakes in January 2003, and their metacercarial infections was examined by the muscle compression and artificial digestion techniques. In the Soyang Lake, 161 metacercariae of *Clonorchis sinensis* (0.35 per fish) were harvested from 459 pond smelts examined. Also, 13 metacercariae of *C. sinensis* (0.43 per fish), 1 of *Metagonimus* sp., 4 of *Echinostoma* sp., 148 of *Centrocestus armatus* and 44 unidentified species were collected from 30 minnows. In the Daechung Lake, 369 metacercariae of *C. sinensis* (3.69 per fish) and 51 unidentified species were recovered from 100 pond smelts. The metacercariae of *C. sinensis* were fed to experimental rats, in which the adult flukes were identified. The pond smelts and minnows collected from the Soyang and Daechung Lakes were verified to be the second intermediate hosts and the sources of human *C. sinensis* infection.

Key words: *Clonorchis sinensis*, liver fluke, *Metagonimus* sp., *Echinostoma* sp., *Centrocestus armatus*, intestinal fluke, *Hypomesus olidus*, *Zacco platypus*, metacercaria, Soyang Lake, Daechung Lake

Various species of freshwater fish are known to be the second intermediate hosts for *Clonorchis sinensis* (Ide, 1935; Joo, 1988; Sohn and Choi, 1997; Nam and Sohn, 2000). The pond smelt *Hypomesus olidus* and the minnow *Zacco platypus* are small freshwater fish, and popular for ice-fishing during the winter season in the Republic of Korea; they actively strut in water during winter, but remain in deep water during the other seasons. Without verification, people simply thought that these fish, particularly the pond smelts, would be free from parasitic infections, since they have a clean, translucent body and live in clear, uncontaminated water. There have been newspaper reports that people enjoy catching these fish at ice-fishing festivals during the winter and eat them uncooked.

However, a paper in Japan (Ide, 1935), and another in the Republic of Korea (Nam and Sohn, 2000), have reported the role of the pond smelts as a second inter-

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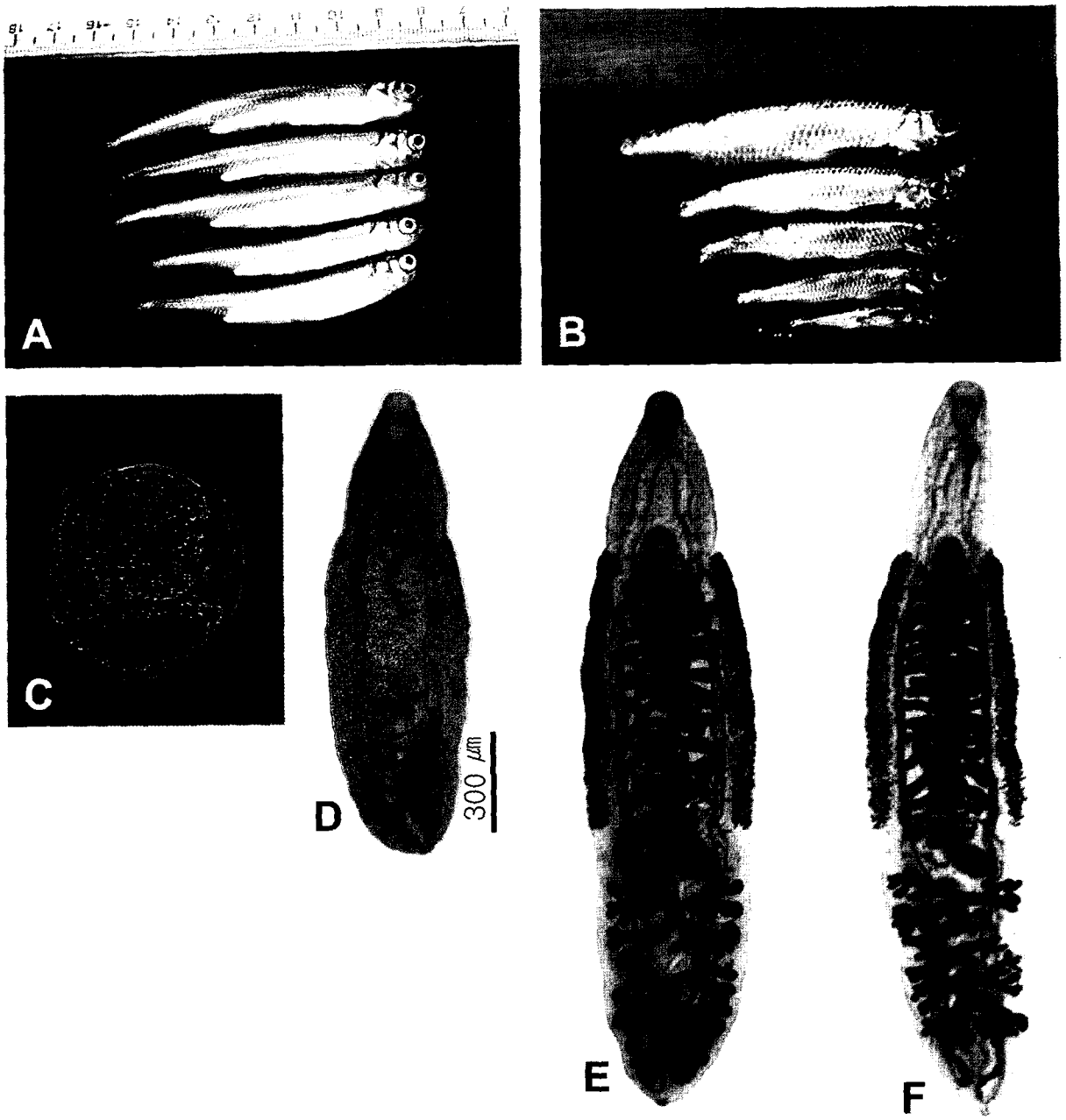

Fig. 1. The pond smelt *Hypomesus olidus* (A) and minnow *Zacco platypus* (B) caught from the Soyang and Daechung Lakes. A metacercaria of *Clonorchis sinensis* (C) isolated from a pond smelt. Adult flukes of *C. sinensis* collected from rats experimentally infected with the metacercariae in pond smelts from the Daechung Lake (D) or Soyang Lake (E) and with metacercariae in minnows from the Soyang Lake (F).

mediate host for *C. sinensis*. According to the latter report, 8 metacercariae of *C. sinensis* were found from a total of 1,305 pond smelts collected from several different localities including the Soyang Lake, Taeho Bay and Paekok Water Reservoir (Nam and Sohn, 2000). With regard to the minnow *Zacco platypus*, natural (Bae et al., 1983) and experimental (Kim, 1964) infections with *C. sinensis* have been reported. Recently, however, there have been no succeeding reports on the metacercarial infections in pond smelts and minnows. Thus, the present study was undertaken to find the status of infections with *C. sinensis* and other

Table 1. Infection status of trematode metacercariae in the pond smelt *Hypomesus olidus* and the minnow *Zacco platypus* during surveys, 2003

Species of fish locality	No. of fish examined	Date of survey	No. of metacercariae detected
Hypomesus olidus			
Soyang Lake	459	Jan. 22-23	*C. sinensis* 161, *Metagonimus* sp. 2, unidentified sp. 22
Daechung Lake	100	Jan. 25	*C. sinensis* 369, unidentified sp. 51
Zacco platypus			
Soyang Lake	30	Jan. 22	*C. sinensis* 13, *Metagonimus* sp. 1, *Echinostoma* sp. 4, *Centrocestus armatus* 148, unidentified sp. 44

trematode metacercariae in pond smelts and minnows in the Soyang and Daechung Lakes.

A total of 559 pond smelts (Fig. 1A) and 30 minnows (Fig. 1B) were collected from the Soyang and Daechung Lakes between 22 and 25 January, 2003 (Table 1). The fish were brought to the laboratory and examined for metacercariae by the artificial digestion and muscle compression techniques. In the digestion technique, the fish were ground in a mortar with a pestle, digested at 37°C for 60 minutes in a 0.6% pepsin-HCl solution, and the metacercariae were sought using a stereomicroscope. In the compression technique, the fish flesh was taken from 4-5 body parts of each fish, compressed between two glass slides, and examined under a stereomicroscope. The metacercariae were identified using light microscopy. However, to confirm the diagnosis, the metacercariae of *C. sinensis* were artificially fed to 8-week-old Sprague-Dawley rats, and the adult flukes harvested at week 4 post-infection (PI).

From 179 pond smelts collected from the Soyang Lake, on January 22, 2003, just before the "Pond smelt festival", organized by a television broadcasting company, a total of 110 metacercariae (0.61 per fish) of *C. sinensis* (Fig. 1C) were detected. To support this observation, the next day, 280 pond smelts were collected, but from a different place of the Soyang Lake, and examined by the same methods. Several species of metacercariae were detected; *C. sinensis* (51 in number; 0.18 per fish), *Metagonimus* sp. (2) and unidentified species (22). Totally, 161 metacercariae of *C. sinensis* were harvested from 459 pond smelts collected from the Soyang Lake (Table 1). From 100 pond smelts collected from the Daechung Lake on January 25, 2003, a total of 369 metacercariae of *C. sinensis* (3.69 per fish) and 51 unidentified species were collected (Table 1).

On the other hand, from 30 minnows (Fig. 1B) collected from the Soyang Lake on 22 January, 2003, a total of 210 metacercariae were collected; *C. sinensis* (13 in total; 0.43 per fish), *Metagonimus* sp. (1), *Echinostoma* sp. (4), *Centrocestus armatus* (148) and an unidentified trematode (44) (Table 1).

Juvenile (Fig. 1D) and adult flukes of *C. sinensis* (Figs. 1E & 1F) were recovered from the bile ducts of the infected rats, with the worm recovery rates of 41.5-92.0% at week 4 PI.

The prevalence of *C. sinensis* infection among the people of the Republic of Korea has decreased since the 1970s (Rim, 1990; Hong et al., 1994). Consequently, a national survey performed in 1997 revealed a 1.4% egg positive rate among 45,832 people random-sampled from all over the country (Ministry of Health and Welfare and Korea Association of Health, 1997). The prevalence of 1.4% for *C. sinensis* was higher than any other kind of helminthiases diagnosed by fecal examinations (Ministry of Health and Welfare and Korea Association of Health, 1997).

It is generally accepted that the prevalence of human clonorchiasis has been decreasing very slowly, irrespective of the remarkable decrease in the ingestion of raw freshwater fish by the younger people in the Republic of Korea. One of the reasons for the relatively steady prevalence includes the long life span of *C. sinensis* in the human bile duct; longer than 10 years (Rim, 1990; Hong, 2003), so infected people can appear as egg positive cases for a long time. However, the possibility of new infections occurring should be

ruled out.

In the present study, the infection rate of the pond smelts with the metacercariae of *C. sinensis* was higher than in the previous reports (Nam and Sohn, 2000). This difference may have been due to the different localities of the very big lake where the fish were caught, or to seasonal differences (Kang et al., 1985; Joo, 1988). The Soyang Lake, for example, is very big, and located in four geographical districts; Inje-gun, Yanggu-gun, Hongchun-gun and Chunchon City. It is noteworthy that the metacercarial burden of *C. sinensis* was higher in the pond smelts caught from the Daechung Lake than those from the Soyang Lake. Further investigations will be required to confirm this result.

It is already known that minnows are an important intermediate host for a variety of human and animal-infecting trematodes. In the present study, the minnows from the Soyang Lake harbored the metacercariae of less than 5 species. In the past, however, metacercariae of *C. sinensis, Exorchis oviformis, Metorchis orientalis, Metorchis taiwanensis, Metagonimus yokogawai, Metacercaria hasegawai, Centrocestus armatus, Echinochasmus japonicus* and *Pseudoxorchis major* were found from minnows (Bae et al., 1983; Rhee et al., 1984; Kim et al., 1987).

The present study has confirmed that both the pond smelts and minnows caught from the Soyang and Daechung Lakes are infected with metacercariae of *C. sinensis*, and could be sources of human infections. To prevent human clonorchiasis, the eating of these fish in the raw state should be avoided.

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