

Fishborne Trematode Metacercariae Detected in Freshwater Fish from Vientiane Municipality and Savannakhet Province, Lao PDR

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Abstract: Freshwater fish from Vientiane Municipality and Savannakhet Province, Lao PDR were examined by the muscle compression and artificial digestion methods to know the infection status with trematode metacercariae. In the fish from Savannakhet, 2 species of metacercariae, *Opisthorchis viverrini* and *Haplorchis taichui*, were detected. *O. viverrini* metacercariae were found in 6 species of fish, *Puntius brevis*, *Hampala dispar*, *Esomus metallicus*, *Mystacoleucus marginatus*, *Puntioplites falcifer*, and *Cyclocheilichthys armatus*. *H. taichui* metacercariae were detected in 3 species of fish, *P. brevis*, *P. falcifer*, and *M. marginatus*. In the fish from Vientiane, 4 species of metacercariae, *O. viverrini*, *H. taichui*, *Haplorchis yokogawai*, and *Centrocestus formosanus*, were detected. Among them, *O. viverrini* metacercariae were found in 7 species of fish, *Onychostoma elongatum*, *C. armatus*, *H. dispar*, *P. brevis*, *Cyclocheilichthys repasson*, *Osteochilus hasseltii*, and *Hypsibarbus lagleri*. The metacercariae of *H. taichui* were detected in 6 species of fish, *C. repasson*, *O. elongatum*, *C. armatus*, *H. dispar*, *Labiobarbus leptocheila*, and *Cirrhinus molitorella*. The metacercariae of *H. yokogawai* were found in 9 species of fish, *C. repasson*, *O. elongatum*, *C. armatus*, *H. dispar*, *Labiobarbus leptocheila*, *O. hasseltii*, *C. molitorella*, *Hypsibarbus wetmorei*, and *H. lagleri*. The metacercariae of *C. formosanus* were detected in 4 species of fish, *C. repasson*, *P. brevis*, *O. hasseltii*, and *C. molitorella*. From these results, it is confirmed that fishborne trematode metacercariae, i.e. *O. viverrini*, *H. taichui*, *H. yokogawai* and *C. formosanus*, are prevalent in various species of freshwater fish from Savannakhet Province and Vientiane Municipality, Lao PDR.

Key words: *Opisthorchis viverrini*, *Haplorchis taichui*, *Haplorchis yokogawai*, *Centrocestus formosanus*, fishborne trematode metacercariae, Vientiane Municipality, Savannakhet Province, Lao PDR

INTRODUCTION

Fishborne trematode (FBT) infections affect the health of more than 40 million people throughout the world and they are particularly prevalent in Asian countries. These parasites provoke a remarkable morbidity to humans and cause serious damages to aquaculture, which is a valuable source of food and employment in developing countries [1,2].

Lao People's Democratic Republic (Lao PDR) is located in the middle of Southeast Asia and bordered by 5 countries, i.e., China, Myanmar, Thailand, Cambodia, and Vietnam. This coun-

try is administratively divided into 18 regions, Vientiane Municipality and 17 Provinces. The Mekong River flows through the whole country from north to south. A quarter of its territory is located in the Mekong River basin. Like Thai people, Laotian people also have unique food habit. Some of them like to eat dishes containing raw freshwater fish, such as 'Koi pla', 'Pla som', and 'Som fak', and easily infect with FBT. Moreover, they have engaged in aquaculture in the fishponds with latrines which can play an important source of contamination [3,4].

It has been revealed that so many Laotians are infected with trematodes such as *Opisthorchis viverrini*, heterophyids, echinostomes, and lecitodendriids by the work of 'Korea-Lao PDR Collaborative Project of Parasite Eradication' [5-7]. Some investigators have previously reported that *O. viverrini* and some sp-

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ecies of intestinal flukes are prevalent as FBT in Lao PDR [8-14]. However, the major source of infections has not been obvious yet. Therefore, we performed epidemiological surveys to know the second intermediate hosts of FBT and the infection status of metacercariae in freshwater fish from Vientiane Municipality and Savannakhet Province, Lao PDR.

MATERIALS AND METHODS

We collected 156 freshwater fish of 17 species from fishermen and small-scale fish dealers in Savannakhet in July 2002 and 177 fish of 12 species in Vientiane in November 2002 (Fig. 1). All collected fish were transferred to the laboratories (local laboratories in Lao PDR and Department of Parasitology and Institute of Health Sciences, Gyeongsang National University College of Medicine). The fish species were identified with the aid of ichthyologists in Lao PDR and FishBase site in internet [15], and individually measured the length and weight according to the species (Tables 1, 2). They all were examined by the artificial digestion method except a few from Savannakhet which were examined by a muscle compression method.

The digenetic trematode metacercariae were segregated and collected from the sediment of digested materials using a stere-

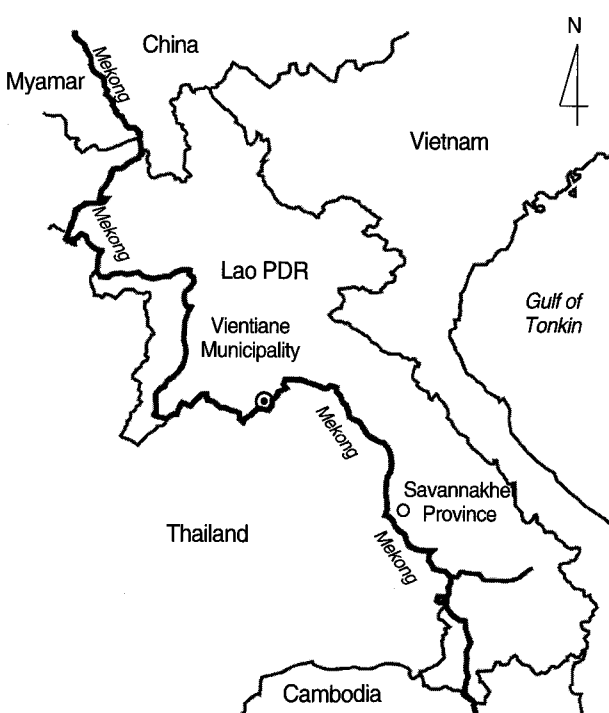


Fig. 1. Surveyed areas, Vientiane Municipality (●) and Savannakhet Province (○), in Lao PDR.

Table 1. Freshwater fishes^a collected from Savannakhet Province, Lao PDR

ST category	No. of fish examined	Length (cm)		Weight (g)	
		Range	Average	Range	Average
Cyprinidae					
<i>Puntius brevis</i>	54	3.5-9.0	6.5	1.0-10.4	5.6
<i>Rasbora tornieri</i>	16	4.0-6.5	5.4	1.0-4.0	2.0
<i>Puntioplites falcifer</i>	14	4.3-13.0	7.6	12.0-16.0	15.1
<i>Hampala dispar</i>	11	10.5-13.0	10.5	14.0-40.0	21.3
<i>Esomus metallicus</i>	10	4.5-6.0	5.0	1.0-3.0	1.7
<i>Sikukia gudgeri</i>	7	7.0-10.0	8.4	6.0-10.0	7.0
<i>Cyclocheilichthys apogon</i>	6	5.0-12.5	11.8	19.0-34.0	25.0
<i>Cyclocheilichthys armatus</i>	6	7.6-9.9	8.5	10.1-22.9	14.5
<i>Rasbora myersi</i>	5	8.0-11.0	10.2	6.0-17.0	11.4
<i>Rasbora dusonensis</i>	5	6.5-8.5	7.5	-	-
<i>Thynnichthys thynnoides</i>	5	9.0-10.5	9.8	-	-
<i>Mystacoleucus marginatus</i>	4	6.5-8.5	7.6	4.0-8.0	6.0
<i>Osteochilus hasseltii</i>	3	11.5-12.0	11.9	22.0-37.0	27.7
<i>Osteochilus lini</i>	2	7.0-9.5	8.3	-	-
Ambassidae					
<i>Parambassis apogonoides</i>	4	-	3.0	-	1.0
Anabantidae					
<i>Anabas testudineus</i>	2	5.0-8.0	6.5	4.0-12.0	8.0
Mastacembelidae					
<i>Macrognathus siamensis</i>	2	-	13.0	-	15.0

^aA total of 156 fish of 17 species were examined.

Table 2. Freshwater fish^a collected from Vientiane Municipality, Lao PDR

Species of fish	No. of fish examined	Length (cm)		Weight (g)	
		Range	Average	Range	Average
Cyprinidae					
<i>Cyclocheilichthys repasson</i>	35	5.0-7.5	6.0	3.1-9.1	5.3
<i>Onychostoma elongatum</i>	34	6.7-12.0	8.4	7.7-38.2	12.2
<i>Puntius brevis</i>	25	5.6-6.2	5.9	2.2-5.6	3.6
<i>Cyclocheilichthys armatus</i>	20	6.2-13.5	8.0	4.5-44.2	13.6
<i>Hampala dispar</i>	18	5.7-12.8	10.7	4.2-51.2	35.8
<i>Labiobarbus leptocheila</i>	11	7.0-8.7	7.9	5.6-11.3	8.8
<i>Osteochilus hasseltii</i>	10	6.0-9.0	7.5	5.8-17.8	10.4
<i>Cirrhinus molitorella</i>	8	5.0-7.2	6.4	2.8-9.1	6.6
<i>Hypsibarbus wetmorei</i>	4	11.5-18.0	13.9	45.8-164.0	84.2
<i>Hypsibarbus lagleri</i>	2	5.5-10.0	7.8	4.7-27.9	16.3
Anabantidae					
<i>Anabas testudineus</i>	5	4.5-7.2	6.5	7.8-18.0	16.2
Osphronemidae					
<i>Trichogaster microlepis</i>	5	-	-	-	4.5

^aA total of 177 fish of 12 species were examined.

omicroscope. The metacercariae collected were categorized according to their size and morphological characters, and then the intensity of infection and the infection rate of each fish species were calculated. To obtain the adult flukes, each species of metacercariae was experimentally infected to a cat and hamsters. The adult flukes were recovered from the experimental

animals at day 10 to day 30 after infection, and identified on the basis of their morphologies to confirm the definite species of metacercariae.

RESULTS

Infection status of fish from Savannakhet Province

Two species of trematode metacercariae, *O. viverrini* and *Haplorchis taichui*, were found in the fish from Savannakhet. The metacercariae of *O. viverrini* were detected in 4 fish species, *Puntius brevis*, *Hampala dispar*, *Esomus metallicus*, and *Mystacoleucus marginatus* by the muscle compression method. They were detected also by the digestion method in 3 fish species, *P. brevis*, *Puntioplites falcifer*, and *Cyclocheilichthys armatus*, with the average number of metacercariae per infected fish of 360, 1.3, and 1,990, respectively (Table 3). The metacercariae of *H. taichui*

Table 3. Infection status of fish with *Opisthorchis viverrini* metacercariae in Savannakhet Province

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Puntius brevis</i>	25	23 (92.0)	8,274	24-1,227	360
	29	25 (86.2)		10-120 ^a	
<i>Hampala dispar</i>	11	8 (72.7)		5-10 ^a	
<i>Puntioplites falcifer</i>	9	3 (33.3)	4	1-2	1.3
<i>Esomus metallicus</i>	8	6 (75.0)		3-15 ^a	
<i>Cyclocheilichthys armatus</i>	6	6 (100)	11,939	8-4,030	1,990
<i>Mystacoleucus marginatus</i>	4	2 (50.0)		2-5 ^a	

^aNo. of metacercariae per a gram of fish flesh.

Table 4. Infection status of fish with *Haplorchis taichui* metacercariae in Savannakhet Province

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Puntius brevis</i>	25	2 (8.0)	9	3-6	4.5
<i>Puntioplites falcifer</i>	9	2 (22.2)	2		1.0
	5	2 (40.0)		1 ^a	
<i>Mystacoleucus marginatus</i>	4	1 (25.0)		1 ^a	

^aNo. of metacercariae per a gram of fish flesh.

Table 5. Infection status of fish with *Opisthorchis viverrini* metacercariae in Vientiane Municipality

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Onychostoma elongatum</i>	9	4 (44.4)	9,761	5-9,500	2,440
	25	-	7,768	-	-
<i>Cyclocheilichthys armatus</i>	20	20 (100)	12,936	47-6,980	647
<i>Hampala dispar</i>	13	13 (100)	5,272	21-1,958	406
	5	-	408	-	-
<i>Puntius brevis</i>	25	-	646	-	-
<i>Cyclocheilichthys repasson</i>	10	8 (80.0)	16	1-6	2.0
	25	-	100	-	-
<i>Osteochilus hasseltii</i>	10	2 (20.0)	2	-	1.0
<i>Hypsibarbus lagleri</i>	2	1 (50.0)	115	-	115

Table 6. Infection status of fish with *Haplorchis taichui* metacercariae in Vientiane Municipality

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Cyclocheilichthys repasson</i>	10	6 (60.0)	62	1-27	10.3
	25	-	21	-	-
<i>Onychostoma elongatum</i>	9	4 (44.4)	26	3-15	6.5
	25	-	22	-	-
<i>Cyclocheilichthys armatus</i>	20	2 (10.0)	35	1-34	17.5
<i>Hampala dispar</i>	13	4 (30.8)	7	1-2	1.8
<i>Labiobarbus leptocheila</i>	11	8 (72.7)	20	1-5	2.5
<i>Cirrhinus molitorella</i>	8	1 (12.5)	1	-	1.0

Table 7. Infection status of fish with *Haplorchis yokogawai* metacercariae in Vientiane Municipality

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Cyclocheilichthys repasson</i>	10	1 (10.0)	5	-	5.0
	25	-	6	-	-
<i>Onychostoma elongatum</i>	9	6 (66.7)	434	2-214	72.0
	20	-	3,314	-	-
<i>Cyclocheilichthys armatus</i>	20	20 (100)	7,090	8-1,370	355
<i>Hampala dispar</i>	13	9 (69.2)	2,012	6-522	224
<i>Labiobarbus leptocheila</i>	11	7 (63.6)	223	2-112	40
<i>Osteochilus hasseltii</i>	10	5 (50.0)	15	1-7	3.0
<i>Cirrhinus molitorella</i>	8	2 (25.0)	4	1-3	2.0
<i>Hypsibarbus wetmorei</i>	4	3 (75.0)	279	61-123	93
<i>Hypsibarbus lagleri</i>	2	2 (100)	246	10-236	123

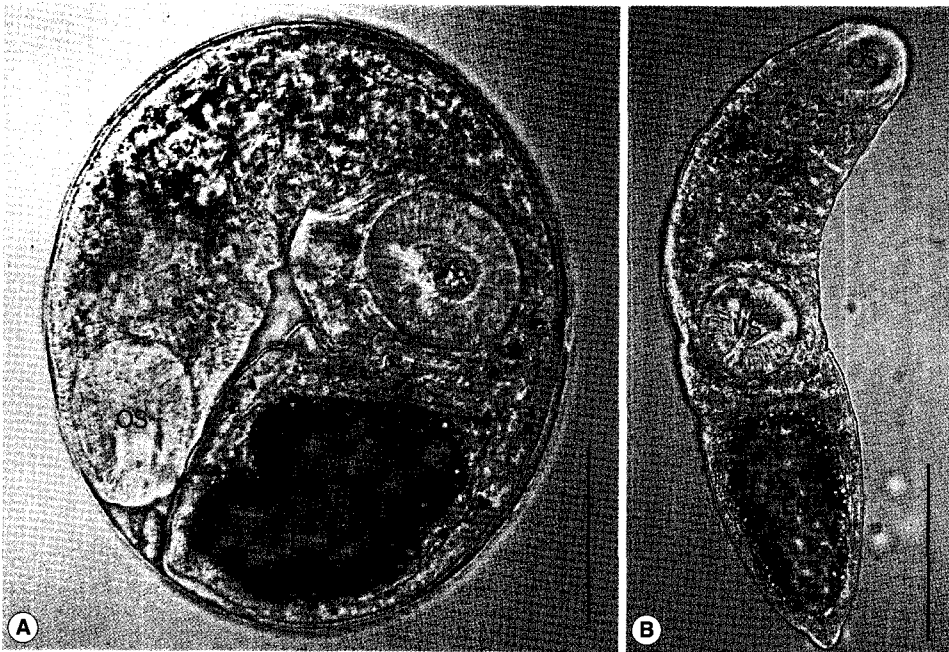


Fig. 2. (A) *Opisthorchis viverrini* metacercaria detected in a freshwater fish from Lao PDR. Scale bar = 75 μ m. (B) An excysted *O. viverrini* metacercaria which has nearly equal sized 2 suckers (OS: oral sucker; VS: ventral sucker), brownish pigment scattered in the body, and a round excretory bladder (EB) in the posterior portion. Scale bar = 150 μ m.

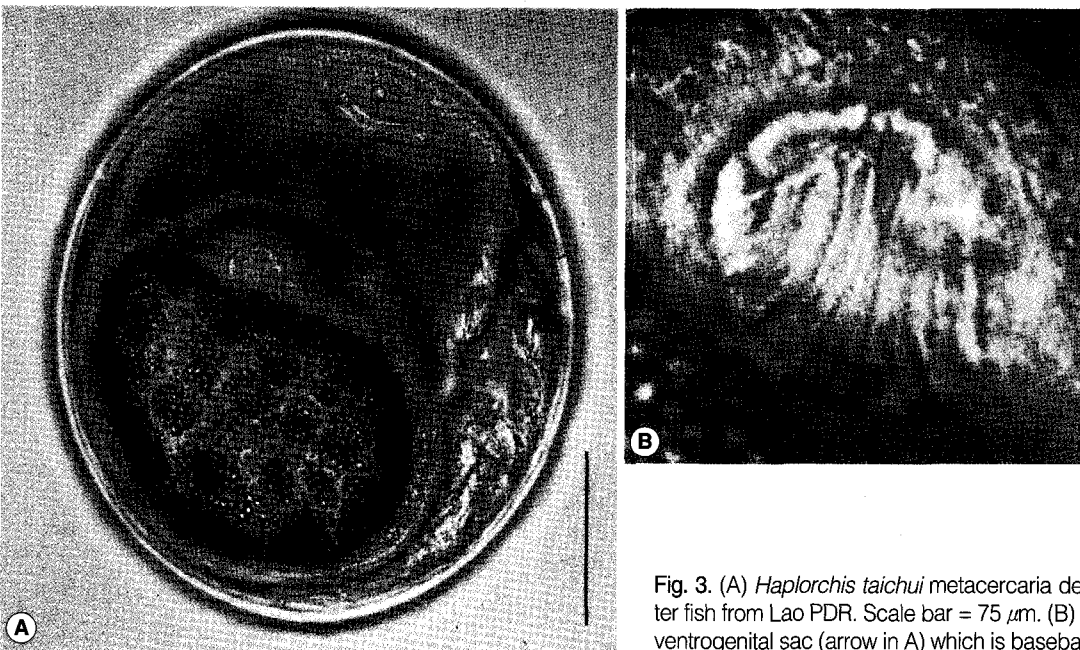


Fig. 3. (A) *Haplorchis taichui* metacercaria detected in a freshwater fish from Lao PDR. Scale bar = 75 μ m. (B) Enlarged view of the ventrogenital sac (arrow in A) which is baseball glove shaped.

were detected in 2 fish species, *P. falcifer* and *M. marginatus*, by the muscle compression method, and also by the digestion method in 2 fish species, *P. brevis* and *P. falcifer* (Table 4).

Infection status of fish from Vientiane Municipality

Four species of trematode metacercariae, *O. viverrini*, *H. taichui*, *Haplorchis yokogawai*, and *Centrocestus formosanus*, were detected in the fish from Vientiane Municipality. Among them, *O. viverrini* metacercariae were detected in 7 fish species, *Ony-*

chostoma elongatum, *Cyclocheilichthys armatus*, *H. dispar*, *P. brevis*, *Cyclocheilichthys repasson*, *Osteochilus hasseltii*, and *Hypsibarbus lagleri*. Their infection rates and densities in each fish species are shown in Table 5.

H. taichui metacercariae were detected in 6 fish species, *C. repasson*, *O. elongatum*, *C. armatus*, *H. dispar*, *Labiobarbus leptocheila*, and *Cirrhinus molitorella*, and their infection rates and densities are shown in Table 6. *H. yokogawai* metacercariae were found in 9 fish species, *C. repasson*, *O. elongatum*, *C. armatus*, *H.*

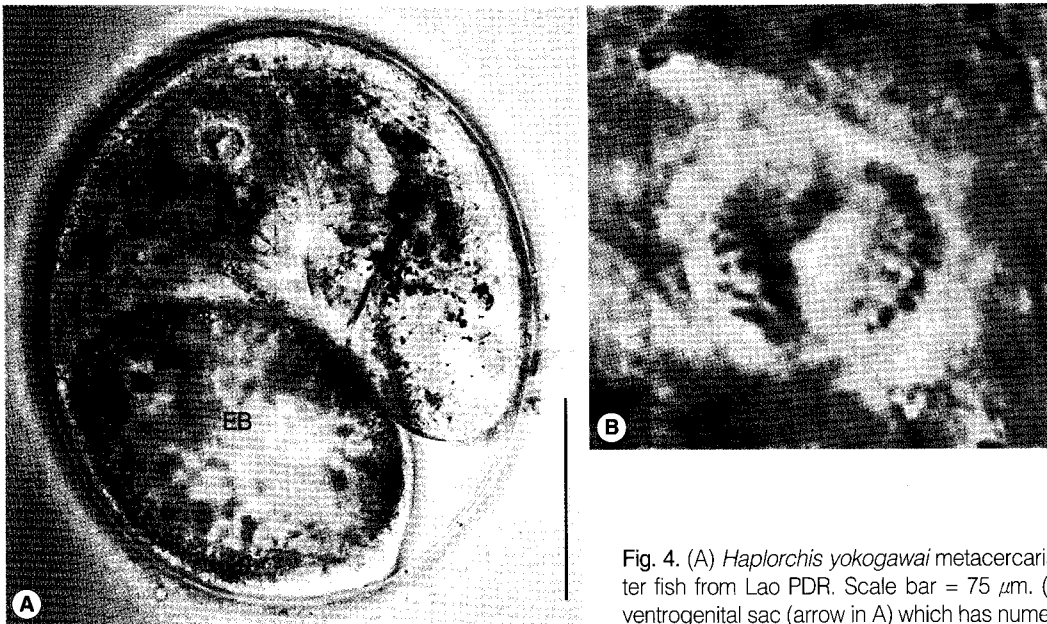


Fig. 4. (A) *Haplorchis yokogawai* metacercaria detected in a freshwater fish from Lao PDR. Scale bar = 75 μ m. (B) Enlarged view of the ventrogenital sac (arrow in A) which has numerous minute spines.

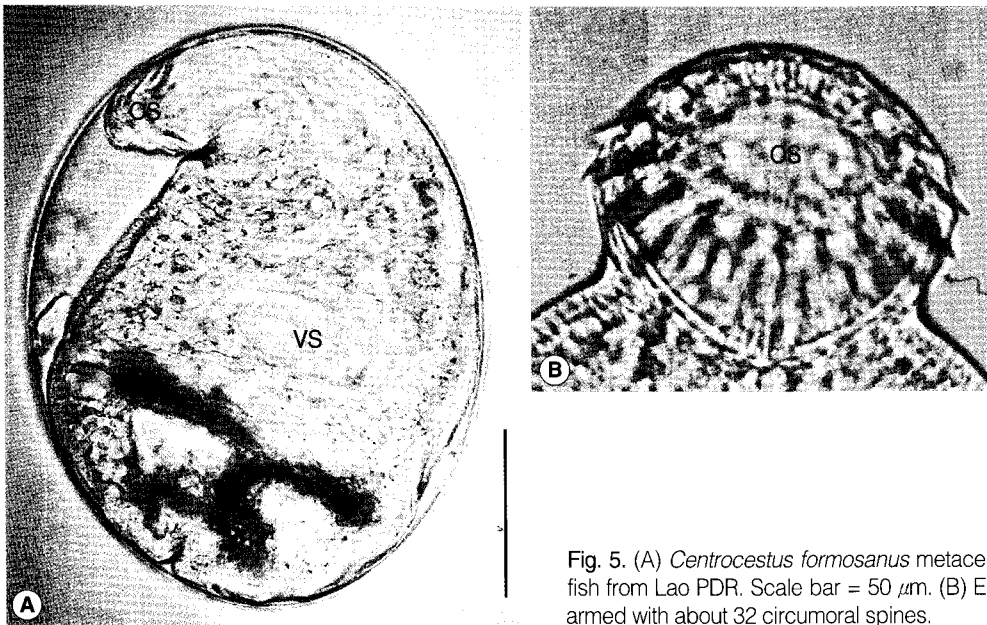


Fig. 5. (A) *Centrocestus formosanus* metacercaria detected in a freshwater fish from Lao PDR. Scale bar = 50 μ m. (B) Enlarged view of the oral sucker armed with about 32 circumoral spines.

Table 8. Infection status of fish with *Centrocestus formosanus* metacercariae in Vientiane Municipality

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Cyclocheilichthys repasson</i>	10	2 (20.0)	7	3-4	3.5
<i>Puntius brevis</i>	25	-	18	-	-
<i>Osteochilus hasseltii</i>	25	-	713	-	-
<i>Osteochilus hasseltii</i>	10	4 (40.0)	39	1-35	9.8
<i>Cirrhinus molitorella</i>	8	2 (25.0)	15	4-11	7.5

dispar, *L. leptocheila*, *O. hasseltii*, *C. molitorella*, *Hypsibarbus wetmorei*, and *H. lagleri*, and their infection rates and densities are revealed in Table 7. *C. formosanus* metacercariae were detected in 4 fish species, *C. repasson*, *P. brevis*, *O. hasseltii*, and *C. molitorella*, and their infection rates and densities are showed in Table 8.

Morphology of metacercariae

Metacercariae of *O. viverrini* were elliptical, 0.19-0.25 \times 0.15-0.22 mm in size, had nearly equal sized 2 suckers, brownish

pigment granules scattered within the body and an O-shaped excretory bladder occupying the greater part of the posterior body (Fig. 2).

Metacercariae of *H. taichui* were elliptical, 0.19-0.22 × 0.16-0.19 mm in size, had a baseball glove-shaped ventro-genital sac with 11-19 chitinous rodlets and an O-shaped excretory bladder occupying the large portion of the posterior body (Fig. 3).

Metacercariae of *H. yokogawai* were elliptical or round, 0.17-0.24 × 0.15-0.23 mm in size, had a U-shaped ventrogenital sac with 70-74 min spines and an O-shaped excretory bladder occupying the large portion of the posterior body (Fig. 4).

Metacercariae of *C. formosanus* were elliptical, 0.15-0.20 × 0.10-0.12 mm in size, had 32 circumoral spines around the oral sucker arranged in 2 rows, and a X-shaped excretory bladder occupying the greater part of the posterior body (Fig. 5).

DISCUSSION

By the present study, it has been confirmed that FBT metacercariae, i.e. *O. viverrini*, *H. taichui*, *H. yokogawai*, and *C. formosanus*, are prevalent in various species of freshwater fish from Savannakhet Province and Vientiane Municipality, Lao PDR. Scholz et al. [16] described 5 species of trematode metacercariae, *O. viverrini*, *H. taichui*, *H. pumilio*, *Stellantchasmus falcatus*, and *C. formosanus*, from freshwater fish in rice fields around Vientiane Municipality and Nam Ngum water reservoir, Lao PDR. Ditrich et al. [9] and Giboda et al. [10] also detected 4 (*O. viverrini*, *H. taichui*, *H. pumilio*, and *S. falcatus*) and 3 (*O. viverrini*, *H. taichui*, and *H. pumilio*) species of trematode metacercariae in the freshwater fish from rice fields around Vientiane Municipality and Nam Ngum water reservoir. However, the data of Ditrich et al. [9] and Giboda et al. [10] are jointly owned by those of Scholz et al. [16]. Therefore, we would like to refer the findings of Scholz et al. [16].

Scholz et al. [16] examined 782 freshwater fish of 45 species collected from rice fields around Vientiane Municipality and Nam Ngum water reservoir for the presence of trematode metacercariae. We also examined 156 freshwater fish of 17 species from Savannakhet and 177 fish of 12 species from Vientiane Municipality. We mainly used the artificial digestion method to detect trematode metacercariae except for a few fish from Savannakhet which were examined by a muscle compression method. However, Scholz et al. [16] examined all fish by the muscle compressive method.

As the infection sources of *O. viverrini*, various species of fresh-

water fish, including *Cyclocheilichthys siaja* (= *C. armatus*), *H. dispar*, *Puntius orphoides*, *Puntius gonionotus* (= *Barbonymus gonionotus*), *Puntius proctozystron* (= *Puntioplites proctozystron*), *Puntius viehoeveri* (= *Barbonymus gonionotus*), *Labiobarbus lineatus*, *Esomus metallicus*, and *Osteochilus* sp., have been reported in Thailand [17,18]. In Lao PDR, 7 fish species, *H. dispar*, *Hampala macrolepidota*, *Barbodes gonionotus* (= *Barbonymus gonionotus*), *C. repasson*, *P. brevis* and 2 *Puntius* spp., were verified to be the second intermediate hosts [16]. While we found *O. viverrini* metacercariae in 6 fish species, *H. dispar*, *P. brevis*, *C. armatus*, *M. marginatus*, *P. falcifer*, and *E. metallicus*, from Savannakhet Province, and in 7 fish species, *H. dispar*, *P. brevis*, *C. armatus*, *C. repasson*, *O. elongatum*, *O. hasseltii* and *H. lagleri*, from Vientiane Municipality. It has been confirmed by the present study that 5 fish species, *M. marginatus*, *P. falcifer*, *O. elongatum*, *O. hasseltii*, and *H. lagleri*, are the new second intermediate hosts for *O. viverrini* among the literature.

The infection rates and intensities of *O. viverrini* metacercariae in fish were much higher in the present study compared with those of Scholz et al. [16]. Especially, 2 fish species, *C. armatus* and *P. brevis*, from Savannakhet Province, and 3 species, *H. dispar*, *C. armatus*, and *O. elongatum*, from Vientiane Municipality were heavily infected with the metacercariae of *O. viverrini*. These fish are highly dangerous if eaten raw by people because of the high density of metacercariae. This liver fluke species can provoke severe pathological changes in the biliary passage, such as dilatation, wall thickening, inflammation and mucosal hyperplasia of the bile duct, and liver cirrhosis [17]. Moreover, this fluke species has been recognized as an important risk factor in the development of cholangiocarcinoma in humans [19,20].

H. taichui metacercariae have been recorded in fish from several Asian countries, i.e. India, China, Taiwan, the Philippines, and Laos [16,21-23]. They were detected in 4 fish species, *Cirrhinus reba* (= *Labeo ariza*), *Amblypharyngodon mola*, *Labeo bata*, and *Puntius sophore*, in India, 3 fish species, *Puntius binotatus*, *Puntius palata* (= *Spratellicypris palata*), and *Ophicephalus striatus* (= *Channa striata*), in the Philippines, 3 fish species, *B. gonionotus*, *P. brevis*, and *Puntius orphoides*, in Thailand, and 4 fish species, *H. dispar*, *H. macrolepidota*, *B. gonionotus*, and *Mystacoleucus maginatus*, in Lao PDR [16,21-24]. In the present study, *H. taichui* metacercariae were detected in 3 fish species, *P. brevis*, *M. marginatus*, and *P. falcifer*, from Savannakhet Province, and in 6 fish species, *H. dispar*, *C. armatus*, *C. repasson*, *O. elongatum*, *L. leptochela*, and *C. molitorella*, from Vientiane Municipality. It has been confirmed by the present study that 6 fish species, *P.*

falcifer, *C. armatus*, *C. repasson*, *O. elongatum*, *L. leptocheila*, and *C. molitorella*, are recorded as new second intermediate hosts of *H. taichui* in the literature.

H. yokogawai metacercariae have been reported in Asian countries such as Taiwan, India, and Thailand, and in Egypt [25]. They were found in 11 fish species, *Mystus vittatus*, *Channa punctatus*, *C. reba* (= *Labeo ariza*), *A. mola*, *L. bata*, *Nandus nandus*, *Puntius chola*, *Puntius sarana*, *P. sophore*, *Glossogobius giurus* (= *G. giuris*), and *Ompok bimaculatus* in India, 6 fish species, *Puntius binotatus*, *P. orphoides*, *Puntius leiacanthus*, *Cyprinus carpio*, *Osteochilus lini*, and *Puntius gonionotus* (= *Barbonymus gonionotus*), in Thailand, and 2 fish species, *Tilapia nilotica* (= *Oreochromis niloticus niloticus*) and *Tilapia zillii*, in Egypt [22,24,26-29]. In the present study, *H. yokogawai* metacercariae were detected in 9 fish species, *H. dispar*, *C. armatus*, *C. repasson*, *O. elongatum*, *L. leptocheila*, *O. hasseltii*, *H. wetmorei*, *H. lagleri*, and *C. molitorella*, from Vientiane Municipality. These 9 fish species have never been reported as the second intermediate hosts for *H. yokogawai* in the literature. Therefore, here we record aforementioned 9 fish species as new second intermediate hosts for *H. yokogawai*.

C. formosanus has been recorded in China, Taiwan, Japan, the Philippines, and India [30,31]. Scholz et al. [16] detected *C. formosanus* metacercariae in only 1 fish species, *Esomus longimana* (= *E. longimanus*), among 45 fish species from rice fields around Vientiane Municipality and Nam Ngum water reservoir, Lao PDR. Recently, Han et al. [32] found *C. formosanus* metacercariae in *P. brevis* fish from Vientiane Municipality, Lao PDR. In the present study, we found them in 4 fish species, *C. repasson*, *P. brevis*, *O. hasseltii*, and *C. molitorella*, from Vientiane Municipality. Three among the 4 fish species, *C. repasson*, *O. hasseltii*, and *C. molitorella*, are added as new second intermediate host for *C. formosanus*.

The eggs of opisthorchiid flukes including *O. viverrini* are indistinguishable from those of heterophyid flukes in Kato-Katz fecal smears. Chai et al. [6,7] tried to recover adult flukes from people to know the exact status of trematode infection in 3 administrative regions of Lao PDR, i.e., Vientiane Municipality, Saravane Province, and Savannakhet Province, after praziquantel treatment and purgation with MgSO₄. In these studies, it was confirmed that *O. viverrini* and *H. taichui* are the 2 most common fluke species, and various fluke species are mixed-infected in the inhabitants. However, the intensity of infection with each fluke species varied by locality surveyed. In the case of *O. viverrini*, the worm burdens were high in Vientiane Municipality and Savannakhet Province, whereas the burden was

comparatively low in Saravane Province. The intensity of *O. viverrini* infection was the highest in Savannakhet Province, followed by Vientiane Municipality, and Saravane Province [6,7]. However, in the case of *H. taichui*, the average number of worms recovered per person was remarkably high in Saravane Province, whereas it was comparatively low in Savannakhet Province and Vientiane Municipality [6,7]. From these findings, it was proved that *O. viverrini* is more endemic in Vientiane Municipality and Savannakhet Province, and *H. taichui* is highly endemic in Saravane Province.

On the other hand, survey data on larval fluke infections in intermediate hosts can also be used as an epidemiological index. In the present study, the metacercariae of *O. viverrini* and *H. taichui* were detected in the fish from Savannakhet, and the metacercariae of *O. viverrini*, *H. taichui*, *H. yokogawai* and *C. formosanus* were detected in the fish from Vientiane. Among them, *O. viverrini* and *H. taichui* were the 2 predominant species in both localities. The metacercarial density was the highest for *O. viverrini* in the fish from the 2 localities. This finding is well corresponded to the report of Chai et al. [6,7]. Conclusively, it has been shown by the present study that various freshwater fish from Vientiane Municipality and Savannakhet Province, Lao PDR will play a role for infection sources of FBT, i.e. *O. viverrini*, *H. taichui*, *H. yokogawai*, and *C. formosanus*.

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