Fishborne Trematode Metacercariae in Luang Prabang, Khammouane, and Saravane Province, Lao PDR

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INTRODUCTION

Fishborne trematode (FBT) infection is a public health problem in some Asian countries, including Lao People’s Democratic Republic (Lao PDR), Vietnam, Cambodia, and Thailand, where these flukes provoke remarkable morbidity and cause serious damages to aquaculture. Human infections by FBTs are almost entirely caused by habitual consumption of raw fish containing infective larvae, i.e., metacercariae. These infections are highly localized depending on the food habits of residents and on the presence of susceptible snail hosts.

Moreover, FBTs show low host-specificity, and many kinds of reservoir hosts can contribute to the maintenance of the life cycle, making it extremely difficult to control these parasite infections in the epidemiological points of view [1,2].

Lao PDR is located in the middle of Southeast Asia, and has the Mekong River flow through the whole country from north to south. Laotian people residing in the Mekong River basin, which occupy a quarter of the territory, have some unique food habits. Some of them like to eat dishes containing raw freshwater fish, and are easily infected with FBT [3,4].

It has been reported that many Laotian people are infected with trematodes such as Opisthorchis viverrini, heterophyids, echinostomats, and lecithodendriids [5-7]. Some investigators have previously reported that FBTs, including O. viverrini, are prevalent in Lao PDR [8-14]. Metacercarial infections were also investigated in fish intermediate hosts from some local areas in Lao PDR by some workers. Schotz et al. [15] surveyed...
freshwater fishes from rice fields around Vientiane Municipality and Nam Ngum water reservoir. Freshwater fish from Savanakhet Province and Vientiane Municipality were examined for their metacercarial infections by Rim et al. [16]. However, studies on the second intermediate hosts of FBT were performed in limited areas and, therefore, studies on more wide areas are needed to know the variety of fish intermediate hosts for FBT in Lao PDR. Therefore, we performed field surveys to determine the infection status of freshwater fish with FBT metacercariae caught in 3 Provinces, i.e., Luang Prabang, Khammouane, and Saravane, in Lao PDR.

**MATERIALS AND METHODS**

We collected a total of 242 freshwater fish of 40 species in local markets of Khammouane Province (81 fish of 14 species) in March 2003, Saravane Province (97 fish of 13 species) in November 2003, and Luang Prabang (59 fish of 16 species) in February 2004 (Fig. 1). All collected fish were transferred to the laboratories (on-the-spot local laboratories in Lao PDR and then to the Department of Parasitology, Gyeongsang National University School of Medicine) and identified the fish

**Table 1. Freshwater fish collected from 3 Provinces of Lao PDR**

| Species of fish       | No. of fish* collected from |
|-----------------------|-----------------------------|
|                       | Luang Prabang | Khammouane | Saravane | Total |
| Cyprinidae            |               |            |          |
| Barboryimus gonionotus| 1             | 5          | 3        | 9     |
| Cosmochilus harmondi  | -             | 5          | -        | 5     |
| Crossochilus oblongus | -             | 6          | -        | 6     |
| Cyclocheilichthys apogon| 4             | -          | -        | 4     |
| Cyclocheilichthys armatus| -            | 4          | -        | 4     |
| Cyclocheilichthys enoplos| -            | -          | 5        | 5     |
| Cyclocheilichthys furcatus| 4             | -          | -        | 4     |
| Cyclocheilichthys repasson| 2             | 4          | -        | 6     |
| Esomus longimanus      | -             | -          | 7        | 7     |
| Esomus metallicus      | -             | -          | 10       | 10    |
| Hampala dispar         | -             | 2          | 19       | 21    |
| Henicorynchus lineatus| 4             | -          | -        | 1     |
| Henicorynchus siamensis| 4             | -          | -        | 4     |
| Hypsibarbus tagleri    | -             | 9          | -        | 9     |
| Hypsibarbus malcolmii  | -             | 5          | -        | 5     |
| Hypsibarbus pierrei    | 3             | -          | 1        | 4     |
| Hypsibarbus wetmorei   | -             | 3          | 1        | 4     |
| Labiobarbus leptochelius| 3             | -          | -        | 3     |
| Labiobarbus siamensis  | 4             | -          | -        | 4     |
| Lobocheilos rhabdoura  | 1             | -          | 1        | 2     |
| Mekongina erythrosplia | -             | 10         | -        | 10    |
| Mystacoleucus ecutypus | 1             | -          | -        | 1     |
| Mystacoleucus greenwayi| -             | -          | 16       | 16    |
| Neolissochilus stracheyi| 2             | -          | -        | 2     |
| Onychostoma fusiforme  | 1             | 7          | -        | 8     |
| Osteochilus vittatus   | -             | 7          | -        | 7     |
| Oxygaster pointoni     | -             | 10         | -        | 10    |
| Paralabuca barroni     | -             | -          | 10       | 10    |
| Poropuntius dearatus   | -             | -          | 6        | 6     |
| Puntioplites proctozysron| -            | -          | 7        | 7     |
| Puntius brevis         | -             | 3          | 10       | 13    |
| Puntius orphoides      | -             | -          | 1        | 1     |
| Rasbora steineri       | -             | 5          | -        | 5     |
| Scaphognathops bandanensis| 7             | -          | -        | 7     |
| Sinibrama melrosei     | 7             | -          | -        | 7     |
| Clupeidae              |               |            |          |
| Tenualosa thibaudeaui  | -             | 4          | -        | 4     |
| Channidae              |               |            |          |
| Channa striata         | -             | 4          | -        | 4     |
| Hemiramphidae          |               |            |          |
| Dermogenys siamensis   | -             | 1          | -        | 1     |
| Balitoridae            |               |            |          |
| Physoschistura meridonalis| 5             | -          | -        | 5     |
| Siluridae              |               |            |          |
| Kryptopterus bicirrhis | -             | 1          | -        | 1     |

*Total 242 freshwater fish (40 species) were collected and examined.

Fig. 1. Surveyed areas, Luang Prabang Province (LPP), Khammouane Province (KP), and Saravane Province (SP), in Lao PDR.
species with the aid of ichthyologists in Lao PDR and FishBase site in internet (Table 1) [17]. Individual fish was finely ground with a mortar with pestle or a grinder, and the ground fish meat was mixed with artificial gastric juice. The mixture was incubated at 36˚C for 2-3 hr. The digested material was filtered through 1 × 1 mm mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined using a stereomicroscope and then the metacercariae of each FBT species were separately collected based on their general features. The collected metacercariae were categorized according to their measurements and morphologic characters, and then the infection rate and the intensity of infection were calculated according to the fish species.

**RESULTS**

**Opisthorchis viverrini metacercariae**

*O. viverrini* metacercariae were detected in 4 fish species (25.0%), i.e., *Neolissochilus stracheyi*, *Cylochelichthys apogon*, *Hypsibarbus pierrei*, and *Cylochelichthys furcatus*, from Luang Prabang. Their infection rate was 38.5% and the average metacercarial density was 88 per infected fish. Total 6 (31.6%) out of 19 fish of 5 species (26.3%), i.e., *Onychostoma fusiforme*, *Puntius brevis*, *Cylochelichthys armatus*, *Hampala dispar*, and *Hypsibarbus wetmorei*, from Khammouane were infected with av. 187 metacercariae per fish. A total of 24 fish (42.1%) of 6 species (46.2%), i.e., *H. dispar*, *Puntioplites proctozysron*, *Poropuntius dearatus*, *P. brevis*, *Paralabuca barroni*, and *Cylochelichthys enoplos*, from Saravane were infected with av. 303 metacercariae per fish. The status of metacercarial infection by surveyed areas and fish species are designated in Table 2.

**Haplorchis taichui metacercariae**

*H. taichui* metacercariae were detected in 10 fish species (62.5%) from Luang Prabang, and their infection rate was 86.4% and the average metacercarial density was 260 per infected fish. Total 23 (79.3%) out of 29 fish of 5 species (26.3%) from Khammouane were infected with av. 1,084 metacercariae per fish. A total of 41 fish (54.7%) of 9 species (64.3%) from Saravane were infected with av. 359 metacercariae per fish. The infection status by surveyed areas and fish species are shown in Table 3.

**Haplorchis yokogawai metacercariae**

*H. yokogawai* metacercariae were detected in 6 fish species (37.5%) from Luang Prabang, and their infection rate was 70.8% and the average metacercarial density was 362 per infected fish. Total 20 (66.7%) out of 30 fish of 5 species (26.3%) from Khammouane were infected with av. 126 metacercariae. A total of 55 fish (74.3%) of 8 species (57.1%) from Saravane were infected with av. 214 metacercariae per fish. The infection status by surveyed areas and fish species are shown in Table 4.

**Centrocestus formosanus metacercariae**

Only 1 *C. formosanus* metacercaria was detected in 1 (25.0%) of 5 *Physoschistura meridionalis* from Luang Prabang. No metacercariae were detected in fish from Khammouane. Total 7 fish (29.2%) of 3 species (21.4%), i.e., *Mystacoleucus greenwayi*, *P. proctozysron*, and *Hypsibarbus pierrei*, from Saravane were infected with av. 3 metacercariae per fish. The infection status by surveyed areas and fish species are shown in Table 5.

**DISCUSSION**

In the present study, mainly 4 species of FBT metacercariae, i.e., *O. viverrini*, *H. taichui*, *H. yokogawai*, and *C. formosanus*,
were detected in freshwater fish from 3 Provinces, i.e., Luang Prabang, Khammouane, and Saravane, in Lao PDR. Schotz et al. [15] found 5 species of trematode metacercariae, *O. viverini*, *H. taichui*, *H. pumilio*, *Stellantchasmus falcatus*, and *C. formosanus*, in freshwater fishes from rice fields around Vientiane Municipality and Nam Ngum water reservoir, Lao PDR [15]. Rim et al. [16] also detected 2 (*O. viverini* and *H. taichui*) and 4 (*O. viverini*, *H. taichui*, *H. yokogawai* and *C. formosanus*) species of metacercariae in freshwater fish from Savannakhet Province and Vientiane Municipality, respectively [16]. On the other hand, Schotz et al. [15] examined 782 freshwater fish of 45 species by the flesh compression method [15]. Rim et al. [16] examined 156 freshwater fish of 17 species in Savannakhet and 177 fish of 12 species in Vientiane Municipality. They mainly used the artificial digestion method with the exception of a few fish from Savannakhet which were examined by the flesh compression method [16]. In the present study, we examined a total of 242 fish of 40 species from Luang Prabang, Khammouane, and Saravane Province by the artificial digestion method.

As the second intermediate hosts for *O. viverini*, 15 species of freshwater fish, i.e., *Barbonymus goniomonotus*, *C. armatus*, *Cyclocheilichthys repasson*, *Esomus metallicus*, *H. dispar*, *Hampala macrolepidota*, *Hypsibarbus lagleri*, *Labiobarbus lineatus*, *Mystacoleucus marginatus*, *Onychostoma elongatum*, *Osteochilus hasseltii*, *Puntioplites falcifer*, *P. proctozystron*, *P. brevis*, and *Puntius orphoides*, have been reported in Thailand [18,19] and Lao PDR [15,16]. In the present study, *O. viverini* metacercariae were detected in 4 fish species, *C. apogon*, *C. furcatus*, *H. pierrei*, and *N. stracheyi*, from Luang Prabang, in 5 fish species, *C. armatus*, *H. dispar*, *H.

### Table 3. Infection status of *Haplorchis taichui* metacercariae by species of fish in 3 Provinces of Lao PDR

| Species of fish                  | No. of fish examined | No. (%) of fish infected | No. of metacercariae detected |
|----------------------------------|----------------------|--------------------------|------------------------------|
|                                  |                      |                          | Total | Range     | Average   |
| Fish from Luang Prabang          |                      |                          |       |           |           |
| *Scaphognathops bandanensis*     | 7                    | 7 (100)                  | 7,095 | 22-3,965  | 1,013.6   |
| *Henicorhynchus siamensis*       | 4                    | 4 (100)                  | 45    | 7-17      | 11.3      |
| *Melogonina erythropsa*          | 10                   | 9 (90.0)                 | 1,317 | 57-308    | 146.3     |
| *Physoschistura meridonialis*    | 5                    | 4 (80.0)                 | 7     | 1-4       | 1.8       |
| *Cyclocheilichthys apogon*       | 4                    | 3 (75.0)                 | 828   | 92-526    | 276.0     |
| *Cyclocheilichthys furcatus*     | 4                    | 3 (75.0)                 | 35    | 1-20      | 11.7      |
| *Sinibrama melrosei*             | 7                    | 5 (71.4)                 | 62    | 1-41      | 12.4      |
| *Mystacoleucus ectypus*          | 1                    | 1 (100)                  | 208   | -         | 208.0     |
| *Barbonymus gonionotus*          | 1                    | 1 (100)                  | 275   | -         | 275.0     |
| *Lobocheilos rhabdoura*          | 1                    | 1 (100)                  | 9     | -         | 9.0       |
| **Subtotal**                     | 44                   | 38 (86.4)                | 9,881 | 1-3,965   | 260.0     |
| Fish from Khammouane             |                      |                          |       |           |           |
| *Hypsibarbus lagleri*            | 9                    | 9 (100)                  | 23,838| 1,149-4,709| 2,649     |
| *Onychostoma fusiforme*          | 7                    | 6 (85.7)                 | 787   | 5-625     | 131.2     |
| *Crossoschelus oblongus*         | 6                    | 5 (83.3)                 | 34    | 3-14      | 6.6       |
| *Cyclocheilichthys repasson*     | 4                    | 1 (25.0)                 | 82    | -         | 82.0      |
| *Puntius brevis*                 | 3                    | 2 (66.7)                 | 185   | 31-154    | 92.5      |
| **Subtotal**                     | 29                   | 23 (79.3)                | 24,926| 3-4,709   | 1,083.7   |
| Fish from Saravane               |                      |                          |       |           |           |
| *Mystacoleucus greenwayi*        | 16                   | 16 (100)                 | 5,394 | 8-1,625   | 337.1     |
| *Hampala dispar*                 | 19                   | 4 (21.1)                 | 6,128 | 16-6,050  | 1,532     |
| *Puntioplites proctozystron*     | 7                    | 5 (71.4)                 | 875   | 2-963     | 175.0     |
| *Poropuntius deareatus*          | 6                    | 5 (83.3)                 | 1,113 | 82-608    | 222.6     |
| *Paralaubuca barroni*            | 10                   | 4 (40.0)                 | 61    | 1-58      | 15.3      |
| *Cyclocheilichthys enoplos*      | 5                    | 3 (60.0)                 | 1,038 | 14-986    | 346.0     |
| *Esomus metallicus*              | 10                   | 2 (20.0)                 | 2     | -         | 1.0       |
| *Lobocheilos rhabdoura*          | 1                    | 1 (100)                  | 91    | -         | 91.0      |
| *Puntius orphoides*              | 1                    | 1 (100)                  | 3     | -         | 3.0       |
| **Subtotal**                     | 75                   | 41 (54.7)                | 14,705| 1-6,050   | 358.7     |
| **Total**                        | 148                  | 102 (68.9)               | 49,512| 1-6,050   | 485.4     |
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wetmorei, O. fusiforme and P. brevis from Khammouane, and 6 fish species, C. enoplos, H. dispar, P. barroni, P. dearatus, P. procto-
zysron, and P. brevis from Saravane Province. Accordingly, 9 fish
species, C. apogon, C. enoplos, C. furcatus, H. pierrei, H. wetmorei,
N. stracheyi, O. fusiforme, P. barroni, and P. dearatus, are newly
recorded as the second intermediate hosts for O. viverrini by
the present study.

The infection rates and intensities of O. viverrini metacercariae
were relatively low in fish from 3 Provinces in the present
study. However, 3 particular fish species, C. apogon from Luang
Prabang, P. brevis from Khammouane, and H. dispar from Saravane Province, were heavily infected with O. viverrini metacercariae. Among them, P. brevis and H. dispar were already known as highly suitable fish hosts for O. viverrini in a previous study [16]. These 3 fish species together with another 2, C. armatus and O. elongatum, are highly dangerous due to the high intensity of metacercariae when they were eaten raw by humans.

The liver flukes, like O. viverrini, provoke severe pathological
changes in the bile duct, such as dilatation, wall thickening, in-
flammation and mucosal hyperplasia, and the cirrhotic chang-
es of the liver [18]. Moreover, they act as a risk factor for devel-
opment of cholangiocarcinoma in humans [20,21].

H. taichui metacercariae have been recorded in fish from

| Table 4. Infection status of Haplorchis yokogawai metacercariae* by species of fish in 3 Provinces of Lao PDR |
| Species of fish | No. of fish examined | No. (%) of fish infected | No. of metacercariae detected |
|-----------------|----------------------|--------------------------|-----------------------------|
| Fish from Luang Prabang | | | |
| Scaphognathops bandanensis | 7 | 6 (85.7) | 1,525 | 4-788 | 254.2 |
| Cyclocheilichthys apogon | 4 | 3 (75.0) | 4,037 | 183-2,874 | 1,345.7 |
| Cyclocheilichthys furcatus | 4 | 3 (75.0) | 443 | 19-312 | 147.7 |
| Sinibrama melrosei | 7 | 3 (42.9) | 21 | 1-17 | 7.0 |
| Mystacoleucus melrosei | 1 | 1 (100) | 128 | - | 128.0 |
| Lobocheilos rhabdoura | 1 | 1 (100) | 4 | - | 4.0 |
| Subtotal | 24 | 17 (70.8) | 6,158 | 1-2,874 | 362.2 |
| Fish from Khammouane | | | |
| Hypsibarbus lagleri | 9 | 9 (100) | 1,387 | 67-274 | 154.1 |
| Onychostoma fusiforme | 7 | 6 (85.7) | 898 | 18-553 | 149.7 |
| Crossocheilus oblongus | 6 | 1 (16.7) | 2 | - | 2.0 |
| Cyclocheilichthys armatus | 4 | 3 (75.0) | 201 | - | 201.0 |
| Cyclocheilichthys repasson | 4 | 1 (25.0) | 29 | - | 17.9 |
| Subtotal | 30 | 20 (66.7) | 2,517 | 2-553 | 125.9 |
| Fish from Saravane | | | |
| Mystacoleucus greenwayi | 16 | 16 (100) | 6,002 | 6-975 | 375.1 |
| Hampala dispar | 19 | 18 (42.1) | 1,249 | 5-1,011 | 156.1 |
| Puntioplites proctozyson | 7 | 6 (85.7) | 210 | 5-120 | 35.0 |
| Paralaubuca barroni | 10 | 9 (90.0) | 1,507 | 173-408 | 251.2 |
| Cyclocheilichthys enoplos | 5 | 5 (100) | 2,516 | 47-2,210 | 503.2 |
| Puntius brevis | 10 | 4 (40.0) | 102 | 1-92 | 25.5 |
| Hypsibarbus pierrei | 1 | 1 (100) | 13 | - | 13.0 |
| Subtotal | 74 | 55 (74.3) | 11,742 | 1-2,210 | 213.5 |
| Total | 128 | 92 (71.9) | 20,417 | 1-2,874 | 221.9 |

*These were encysted chiefly in the fin and scale of fish.

| Table 5. Infection status of Centrocestus formosanus metacercariae by species of fish in 2 Provinces of Lao PDR |
| Species of fish | No. of fish examined | No. (%) of fish infected | No. of metacercariae detected |
|-----------------|----------------------|--------------------------|-----------------------------|
| Fish from Luang Prabang | | | |
| Physoschistura meridionalis | 5 | 1 (25.0) | 1 | - | 1.0 |
| Fish from Saravane | | | |
| Mystacoleucus greenwayi | 16 | 4 (25.0) | 17 | 1-8 | 4.3 |
| Puntioplites proctozyson | 7 | 2 (28.6) | 4 | 1-3 | 2.0 |
| Hypsibarbus pierrei | 1 | 1 (100) | 1 | - | 1.0 |
| Subtotal | 24 | 7 (29.2) | 22 | 1-8 | 3.1 |
| Total | 29 | 8 (27.6) | 23 | 1-8 | 2.9 |
some Asian countries, i.e., India, China, Tailand, the Philippine, and Lao PDR. They have been detected in 35 fish species, i.e., Abbottina rivularis, Amblyphysogodon mola, B. gonionotus, Carassius auratus, Channa striata, Chondrichthys dabrhy, Cirrhinus molitorella, Culter recurvipes, C. armati, C. repasson, Cyprinus carpio, H. dispar, H. macroepilota, Hemibarbus mucates, Hemiculter leuciscus, Hypophthalmichthys molitrix, L. leptocheila, Labeo ariza, Labeo bata, Metria lineata, M. marginatus, O. elongatum, Opsariichthys bidens, Pseudohemeliter dispar, P. falcifer, Puntius binotatus, P. brevis, P. orphoides, Puntius semiiasciulatus, Puntius sophore, Saragogobio dabrhy, Spratellopyris palata, Squalius argentatus, and Toxabramis houdemeri, in endemic countries [15, 16, 22-26]. In the present study, H. taichui metacercariae were found in 9 fish species, B. gonionotus, C. apogon, C. furcatus, Henicorhynchus siamensis, L. rhadouara, Mekongina erythropila, Physoschistura meridionalis, Scaphognathoth bandanensis, and Sinibrama melrosei, from Luang Prabang, in 5 fish species, Crossochelus oblongus, C. repasone, H. lagleri, O. fusiforme, and P. brevis, from Khammouane, and 9 fish species, C. enoplos, E. metallicus, H. dispar, L. rhadouara, Mystacoleucus greenwayi, P. barroni, P. dearatus, P. proctozysron, and P. orphoides, from Saravane Province. Therefore, 13 fish species, C. oblongus, C. apogon, C. enoplos, C. furcatus, E. metallicus, H. lagleri, H. siamensis, L. rhadouara, M. erythropila, M. greenwayi, P. meridionalis, S. bandanensis, and S. melrosei, are added as new second intermediate hosts for H. taichui by the present study.

The infection status of H. taichui metacercariae was relatively high in fish from all 3 Provinces. Infection rates of fish from Luang Prabang (86.4%) and Khammouane (79.3%) were very high, and that of fish from Saravane (54.7%) was moderate. Intensities of H. taichui metacercariae per infected fish were 260, 1,084, and 359 in fish from Luang Prabang, Khammouane, and Saravane Province, respectively. Particularly 3 fish species, S. bandanensis from Luang Prabang, H. lagleri from Khammouane, and H. dispar from Saravane Province, were heavily infected with H. taichui metacercariae, more than 1,000 in number. On the other hand, Chai et al. [6] demonstrated that the worm burden of H. taichui was remarkably high in residents of Saravane Province [6]. Based on these findings and high infection rates and intensities of H. taichui metacercariae in the present study, we could speculate that residents in Khammouane and Luang Prabang may be highly infected with H. taichui like Saravane.

Distribution of H. yokogawai metacercariae has been reported in some Asian countries such as India, Thailand, and Lao PDR. They were found in 28 fish species, A. mola, B. gonionotus, Channa punctatus, C. molitorella, C. armatus, C. repasson, Cyprinus carpio, Glossogobius giuris, H. dispar, Hypsibarbus wutemorei, H. lagleri, Labeo ariza, L. bata, L. leptocheila, Mystus vittatus, Nandus nandus, Ompok bimaculatus, Oreochromis niloticus niloticus, O. elongatum, O. hasseltii, Osteochilus lini, P. binotatus, Puntius chola, Puntius leacamantus, P. orphoides, P. sarana, P. sophore, and Tilapia zillii [16, 23, 25, 27-31]. In the present study, H. yokogawai metacercariae were detected in 6 fish species, C. apogon, C. furcatus, L. rhadouara, M. ectypus, S. bandanensis, and S. melrosei, from Luang Prabang, 5 fish species, C. oblongus, C. armatu, C. repasson, H. lagleri and O. fusiforme, from Khammouane, and 8 fish species, C. enoplos, H. dispar, H. pierrei, M. greenwayi, P. barroni, P. dearatus, P. proctozysron, and P. brevis, from Saravane. Among them, 15 fish species, i.e., C. oblongus, C. apogon, C. enoplos, C. furcatus, H. pierrei, L. rhadouara, M. ectypus, M. greenwayi, O. fusiforme, P. barroni, P. dearatus, P. proctozysron, and P. brevis, S. bandanensis and S. melrosei, have never been reported as the second intermediate hosts of H. yokogawai in the available literature. Therefore, we corded aforementioned 15 fish species as new second intermediate hosts for H. yokogawai.

C. formosanus is distributed in China, Taiwan, Japan, the Philippines, India, and Lao PDR [15, 16, 32-34]. Schotz et al. [15] detected C. formosanus metacercariae in only 1 fish species, Esomus longimanus, among 45 fish species examined. Rim et al. [16] found them in 4 fish species, C. repasson, P. brevis, O. hasseltii, and C. molitorella, in Vientiane Municipality. In the present study, C. formosanus metacercariae were detected in 1 fish species, P. meridionalis, from Luang Prabang, and 3 fish species, M. greenwayi, P. proctozysron, and H. pierrei, from Saravane. Collectively, total 9 fish species, C. molitorella, C. repasson, E. longimanus, H. pierrei, M. greenwayi, O. hasseltii, P. proctozysron, and P. brevis, are listed as the second intermediate hosts for C. formosanus in Lao PDR.

It has been proved that O. viverrini is more endemic in humans and fish in Vientiane Municipality and Savannakhet Province, and H. taichui is highly endemic in humans and fish in Saravane Province [6, 7, 16]. From this study, it is suggested that Luang Prabang and Khammouane are highly endemic areas of intestinal flukes, H. taichui and H. yokogawai, rather than O. viverrini.

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