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Fishborne Trematode Metacercariae in Freshwater Fish from Guangxi Zhuang Autonomous Region, China

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Abstract: A survey was performed to investigate the infection status of fishborne trematode (FBT) metacercariae in freshwater fish from Guangxi Zhuang Autonomous Region, China. A total of 307 freshwater fish of 31 species were collected from 5 administrative regions of Guangxi Zhuang Autonomous Region. They were examined by artificial digestion method from July 2003 to August 2004. No metacercariae were detected in fish from Fusui-xian. In fish from Mashan-xian and a market in Nanning, 3 species of metacercariae, *Haplorchis taichui, Haplorchis pumilio,* and *Centrocestus formosanus,* were mainly detected. Metacercariae (8 in number) of *Clonorchis sinensis* were found in 1 *Chanodichthys dabryi* purchased from a market in Nanning. In fish from Yangshuo, *Metagonimus yokogawai* metacercariae were detected from all 18 fish species examined. Total 13 *C. sinensis* metacercariae were found in 3 out of 10 *Hemibarbus maculatus* from Yangshuo. All 7 *Zacco platypus* from Yangshuo were infected with 8-112 *Echinochasmus perfoliatus* metacercariae. In fish from Binyang-xian, *H. pumilo* metacercariae were mainly detected in all 5 fish species examined, and only 1 metacercaria of *C. sinensis* was found in a *Hemiculter leucisculus*. From the above results, it was confirmed that some species of freshwater fish play a role of second intermediate hosts for FBT in Guangxi Zhuang Autonomous Region, China. In particular, 4 species of intestinal flukes, *M. yokogawai, H. taichui, H. pumilio,* and *C. formosanus,* were prevalent in fish hosts, whereas *C. sinensis* metacercariae were detected only in 3 fish species.

Key words: *Haplorchis taichui, Haplorchis pumilio, Centrocestus formosanus, Metagonimus yokogawai, Clonorchis sinensis,* fishborne trematode metacercariae, freshwater fish, Guangxi Zhuang Autonomous Region, China

INTRODUCTION

Fishborne trematode (FBT) infections affect the health of more than 18 million people around the world, particularly in Asian countries. These flukes provoke remarkable morbidity and cause serious damage to aquaculture in developing countries [1,2]. The liver flukes, *Clonorchis sinensis* and *Opisthorchis* spp., have been known as the representative FBT. Many species of intestinal flukes, mainly belonging to the family Heterophyidae and Echinostomatidae are also infected by eating raw fish [2].

In the Republic of Korea, many species of FBT including *C. sinensis* have been reported. *C. sinensis* is still prevalent in riverside areas and is the most important helminth species of public health importance. About 10 species of the Heterophyidae and 3 of the Echinostomatidae have been reported as the intestinal flukes infected by eating raw fish meat [3-6]. On the other hand, *C. sinensis* infections are found in 22 out of 30 PRM (Province/Autonomous Region/Municipality) in China. Especially, prevalences in 4 provinces, Guangdong, Guangxi, Anhui and Heilongjiang, are much higher than those of the other regions [7-9]. As the intestinal FBT, 8 species of the Heterophyidae (*Metagonimus yokogawai, M. takahashii, Stellantchasmus falcatus, Centrocestus formosanus, Haplorchis pumilio, H. taichui,* and *H. yokogawai*) and 5 species of the Echinostomatidae (*Echinostoma angustitestis, Echinochasmus japonicus, E. perfoliatus, E. liliputanus,* and *E. fujianensis*) are distributed in China [10].

Guangxi Zhuang Autonomous Region (GZAR) locates in the southern part of China, on the subtropical zone and its southwest area contact with Vietnam. This province has many minority people. They have some different food habit. Some monority people like to eat raw fish and easily infect with FBT. Furthe-

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rmore, they have engaged in aquaculture in the fishponds with latrines that contribute as an important source of contamination [11]. It has been revealed that many minority peoples are infected with FBT including *C. sinensis* in GZAR, China [11]. However, the major source of infection has not yet been obvious. Therefore, we performed epidemiological surveys to know the second intermediate hosts and the infection status of FBT metacercariae in freshwater fish from 5 administrative regions of GZAR, China.

MATERIALS AND METHODS

A total of 307 freshwater fish of 31 species was collected from 5 administrative regions, Fusui-xian, Mashan-xian, Nanning City, Yangsuo County, and Binyang-xian, in GZAR, China from July 2003 to August 2004 (Table 1). All collected fish were transferred to the laboratories (Division of Helminthiasis Prevention and Control, Guangxi Centre for Disease Prevention and Control, Nanning, China, and Department of Parasitology, Gyeongsang National University School of Medicine, Jinju, Korea), individually measured the length and weight according to the species, and identified the fish species with the aid of ichthyologists in Nanning, GZAR, China, and FishBase site in internet [12]. They all were finely ground with a mortar with pestle or a grinder. The ground fish meat was mixed with artificial gastric juice and the mixture was incubated at 36℃ for 2-3 hr. The digested material was filtered with 1 × 1 mm of mesh, and washed with 0.85% saline untill the supernatant is clear. The sediment was carefully examined under a stereomicroscope. The metacercariae were separately collected by the general feature, and they were identified species level based on the detail morphologies and their demensions under a light microscope. Identified metacercariae

Table 1. Freshwater fishes collected from 5 localities in Guangxi Zhuang Autonomous Region, China

Species of fish	No. of fish examined in					
	Fusui	Mashan	Nanning	Yangsuo	Binyang	Total
Hemibarbus maculatus	–	4	11	10	–	25
Acheilognathus tonkinensis	–	2	10	6	7	25
Zacoo platypus	–	–	–	22	–	22
Carassius auratus	–	2	11	4	4	21
Hypophthalmichthys molitrix	4	1	13	–	–	18
Acrossocheilus hemispinus	–	–	–	15	–	15
Discogobio tetrabarbatus	–	–	–	14	–	14
Hemiculter leucisculus	–	–	3	1	10	14
Ctenopharyngodon idella	2	–	–	–	10	12
Pseudohemiculter dispar	–	10	–	1	–	11
Opsariichythys bidens	–	8	–	3	–	11
Puntius semifasciolatus	–	4	–	7	–	11
Chanodichthys erythropterus	10	–	–	–	–	10
Microphysogobio fukiensis	–	10	–	–	–	10
Toxabramis houdemeri	–	–	10	–	–	10
Abbottina rivularis	–	–	4	5	–	9
Cyprinus carpio	1	–	4	2	–	7
Squalidus argentatus	–	–	7	–	–	7
Metzia lineata	2	2	–	–	3	7
Cirrhinus molitorella	7	–	–	–	–	7
Sinibrama macrops	–	–	–	6	–	6
Pseudogobio esocinus	–	–	–	5	–	5
Aristichthys nobilis	3	–	–	–	–	3
Culter recurviceps	1	–	2	–	–	3
Squalidus wolterstorffi	–	–	–	2	–	2
Pseudorasbora parva	1	1	–	–	–	2
Saurogobio dabryi	–	–	2	–	–	2
Chanodichthys dabryi	–	–	1	–	1	2
Ancherythroculter lini	–	–	–	1	–	1
Oreochromis niloticus niloticus	7	1	3	–	–	11
Coreoperca whiteheadi	–	–	–	4	–	4
Total	38	45	81	108	35	307

were experimentally infected to mice, rats, hamsters, chicks and cats to obtain adult worms.

RESULTS

Infection status in fish from Fusui-xian and Mashan-xian

No metacercariae were detected in fish from Fusui-xian. However, in fish from Mashan-xian, 3 species of metacercariae, *H. taichui, H. pumilio,* and *C. formosanus,* were detected. *H. taichui* metacercariae were found in 7 fish species and their infection status is presented in Table 2. *H. pumilio* metacercariae were detected in 10 fish species. Among them, *Opsariichthys bidens, Puntius semifasciolatus, Pseudohemiculter dispar,* and *Carassius auratus* were heavily infected (Table 3). *C. formosanus* metacercariae were found in 3 fish species. Especially, *Microphysogobio fukiensis* was infected with more than 300 metacercariae of *C. formosanus* (Table 4).

Table 2. Infection status of *Haplorchis taichui* metacercariae by the species of fish from a stream in Mashan-xian

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Pseudohemiculter dispar	10	10 (100)	250	1-200	25.0
Opsariichthys bidens	8	8 (100)	224	4-82	28.0
Puntius semifasciolatus	4	–	9	–	–
Carassius auratus	2	2 (100)	39	2-37	19.5
Metzia lineata	2	1 (50.0)	5	–	5.0
Hypophthalmichthys molitrix	1	1 (100)	13	–	13.0

Table 3. Infection status of *Haplorchis pumilio* metacercariae by the species of fish from a stream in Mashan-xian

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Microphysogobio fukiensis	10	6 (60.0)	58	1-22	9.7
Pseudohemiculter dispar	10	10 (100)	1,960	30-818	196.0
Opsariichthys bidens	8	8 (100)	3,328	150-1,096	416.0
Hemibarbus maculatus	4	4 (100)	151	25-45	37.8
Puntius semifasciolatus	4	–	886	–	–
Carassius auratus	2	2 (100)	368	20-348	184.0
Metzia lineata	2	2 (100)	217	40-177	108.5
Hypophthalmichthys molitrix	1	1 (100)	85	–	85.0
Pseudorasbora parva	1	1 (100)	4	–	4.0

Table 4. Infection status of *Centrocestus formosanus* metacercariae by the species of fish from a stream in Mashan-xian

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Microphysogobio fukiensis	10	10 (100)	3,980	64-1,375	398.0
Metzia lineata	2	1 (50)	21	–	21.0
Pseudorasbora parva	1	1 (100)	1	–	1.0

Infection status in fish from Nanning City

In freshwater fish from a market in Nanning, 3 species of metacercariae, *H. taichui, H. pumilio*, and *C. formosanus*, were mainly detected. *H. taichui* metacercariae were found in 11 fish species. Among them, *Hemiculter leucisculus, Toxabramis houdemeri,* and *Cyprinus carpio* were heavily infected with *H. taichui*

Table 5. Infection status of *Haplorchis taichui* metacercariae by the species of fish from a market in Nanning City

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Hypophthalmichthys molitrix	13	13 (100)	691	7-159	53.2
Carassius auratus	11	11 (100)	306	4-120	27.8
Hemibarbus maculatus	11	10 (90.9)	401	6-92	40.1
Toxabramis houdemeri	10	10 (100)	1,930	1-485	193.0
Squalidus argentatus	7	3 (42.9)	45	5-20	15.0
Cyprinus carpio	4	3 (75.0)	589	1-356	196.3
Abbottina rivularis	4	2 (50.0)	14	2-12	7.0
Hemiculter leucisculus	3	3 (100)	716	105-396	238.7
Culter recurviceps	2	2 (100)	98	46-52	49.0
Saurogobio dabryi	2	2 (100)	130	35-95	65.0
Chanodichthys dabryi	1	1 (100)	55	–	55.0

Table 6. Infection status of *Haplorchis pumilio* metacercariae by the species of fish from a market in Nanning City

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Hypophthalmichthys molitrix	13	12 (92.3)	748	14-153	62.3
Carassius auratus	11	11 (100)	719	16-253	65.4
Hemibarbus maculatus	11	11 (100)	289	11-52	26.3
Acheilognathus tonkinensis	10	9 (90.0)	597	27-172	66.3
Toxabramis houdemeri	10	7 (70.0)	131	5-32	18.7
Squalidus argentatus	7	7 (100)	272	3-78	38.9
Cyprinus carpio	4	2 (75.0)	380	68-312	190.0
Hemiculter leucisculus	3	3 (100)	183	14-124	61.0
Saurogobio dabryi	2	2 (100)	188	88-100	94.0
Culter recurviceps	2	2 (100)	35	15-20	17.5
Chanodichthys dabryi	1	1 (100)	12	–	12.0

metacercariae (Table 5). *H. pumilio* metacercariae were detected in 11 fish species, and their infection status is shown in Table 6. *C. formosanus* metacercariae were found in 7 fish species and their infection status is shown in Table 7. One *Chanodichthys dabryi* was infected with 3 metacercariae of *C. sinensis*. Four metacercariae of *Metagonimus* sp. were detected in 1 *Hemibarbus maculatus*.

Infection status in fish from Yangshuo County

In freshwater fish from Yangshuo, *Metagonimus* sp. metacercariae were detected from all 18 fish species examined. Among them, *Zacco platypus, H. maculatus, P. semifasciolatus,* and *Abbottina rivularis* were infected with more than 100 metacercariae per infected fish (Table 8). A total of 13 *C. sinensis* metacercariae were found in 3 out of 10 *Hemibarbus maculatus* examined. All 7 *Z. platypus* examined were infected with 8-112 metacercariae of *E. perfoliatus*.

Table 7. Infection status of *Centrocestus formosanus* metacercariae by the species of fish from a market in Nanning City

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Acheilognathus tonkinensis	10	5 (50.0)	29	1-21	5.8
Squalidus argentatus	7	5 (71.4)	62	2-32	12.4
Cyprinus carpio	4	3 (75.0)	15	1-12	5.0
Hypophthalmichthys molitrix	4	3 (75.0)	17	2-13	5.7
Abbottina rivularis	4	4 (100)	56	7-22	14.0
Hemiculter leucisculus	3	1 (33.3)	55	–	55.0
Saurogobio dabryi	1	1 (100)	2	–	2.0

Table 8. Infection status of *Metagonimus yokogawai* metacercariae by the species of fish collected from Yangshuo County

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Zacco platypus	22	22 (100)	9,880	23-1,836	449.1
Acrossocheilus hemispinus	15	11 (73.3)	77	2-36	7.0
Discogobio tetrabarbatus	14	12 (85.7)	64	1-18	5.3
Hemibarbus maculatus	10	10 (100)	2,484	14-731	248.4
Puntius semifasciolatus	7	5 (71.4)	967	87-293	193.4
Sinibrama macrops	6	6 (100)	123	10-30	20.5
Acheilognathus tonkinensis	6	2 (33.3)	5	1-4	2.5
Abbottina rivularis	5	5 (100)	559	14-311	111.8
Pseudogobio esocinus	5	1 (20.0)	32	–	32.0
Carassius auratus	4	1 (25.0)	1	–	1.0
Coreoperca whiteheadi	4	1 (25.0)	2	–	2.0
Hemiculter sp.	4	3 (75.0)	224	5-209	74.7
Opsariichthys bidens	3	3 (100)	114	30-45	38.0
Squalidus wolterstorffi	2	2 (100)	31	3-28	15.5
Cyprinus carpio	2	1 (50.0)	13	–	13.0
Ancherythroculter lini	1	1 (100)	26	–	26.0
Pseudohemiculter dispar	1	1 (100)	31	–	31.0
Hemiculter leucisculus	1	1 (100)	34	–	34.0

Infection status in fish from Binyang-xian

In freshwater fish from Binyang-xian, *H. pumilo* metacercariae were mainly detected in all 5 fish species examined, and their infection status is presented in Table 9. *Metorchis taiwanensis* metacercariae were found in 4 *Ctenopharyngodon idella* (40%) and their numbers were ranged in 1-425. Only 1 metacercaria of *C. sinensis* was detected in *H. leucisculus*.

Overall infection status of fish from GZAR, China

Metacercariae of *Metagonimus* sp. were detected in 18 species of fish, and their fish hosts are revealed in Table 8 (see full names of fish). Those of *H. taichui* were found in 15 fish species, such as *H. molitrix, C. auratus, H. maculatus, T. houdemeri, P. dispar, O. bidens, S. argentatus, C. carpio, A. rivularis, P. semifasciolatus, H. leucisculus, C. recurviceps, S. dabryi, M. lineate,* and *C. dabryi*. *H. pumilio* metacercariae were detected in 18 fish species, i.e., *C. auratus, A. tonkinensis, H. maculatus, H. molitrix, H. leucisculus, C. idella, T. houdemeri, M. fukiensis, P. dispar, O. bidens, S. argentatus, M. lineata, C. carpio, P. semifasciolatus, S. dabryi, C.*

Table 9. Infection status of *Haplorchis pumilio* metacercariae by the species of fish from Binyang-xian

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Hemiculter leucisculus	10	10 (100)	206	5-44	20.6
Ctenopharyngodon idella	10	10 (100)	712	22-144	71.2
Acheilognathus tonkinensis	7	7 (100)	732	68-150	104.6
Carassius auratus	4	4 (100)	334	42-125	83.5
Metzia lineata	3	3 (100)	62	17-23	20.7

Table 10. Fish hosts infected with more than 100 metacercariae per fish by the trematode species

Species of flukes	No. of fish host	Fish hosts infected with more than 100 metacercariae/fish
Metagonimus yokogawai	18	*Z. platypus, A. rivularis, H. maculatus, P. semifasiolatus*
Haplorchis taichui	15	*H. leucisculus, C. carpio, T. houdemeri*
Haplorchis pumilio	18	*C. auratus, C. carpio, A. tonkinensis, O. bidens, M. lineata, P. dispar, P. semifasiolatus*
Centrocestus formosanus	10	*M. fukiensis*

recurviceps, *C. dabryi*, and *P. parva*. *C. formosanus* metacercariae were found in 10 fish species, such as *M. fukiensis*, *A. tonkinensis*, *S. argentatus*, *C. carpio*, *H. molitrix*, *A. rivularis*, *H. leucisculus*, *M. lineata*, *S. dabryi*, and *P. parva* (Table 10). On the other hand, metacercariae of *C. sinensis* were demonstrated in low infection rates and intensities in only 3 fish species, *H. maculatus*, *C. dabryi*, and *H. leucisculus*.

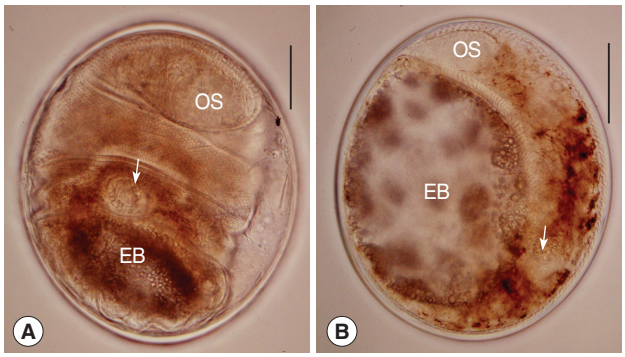

Fig. 1. (A) *Haplorchis taichui* metacercaria detected in a freshwater fish, *Hemiculter leucisculus*, from a market of Nanning City. It is elliptical, has an oral sucker (OS), a baseball glove-shaped ventrogenital sac (arrow mark) with 11-18 rodlets and an O-shaped excretory bladder (EB). Scale bar = 50 μm. (B) *Haplorchis pumilio* metacercaria detected in a freshwater fish, *Opsariichthys bidens*, from Mashan-xian. It is elliptical, has an OS, a ventrogenital complex with 36-42 deer horn-like minute spines, and an O-shaped EB occupying large portion of posterior body. Scale bar = 50 μm.

Morphology of metacercariae detected

H. taichui metacercariae (n = 20) were elliptical, 188-220 (203 in average) × 155-185 (168 in average) μm in size, had a baseball glove-shaped ventro-genital sac with 11-18 rodlets and an O-shaped excretory bladder occupying large portion of posterior body (Fig. 1A).

H. pumilio metacercariae (n = 20) were elliptical, 155-188 (172) × 138-163 (152) μm in size, had 36-42 deer horn-like minute spines arranged in 1-2 rows around ventrogenital complex, and an O-shaped excretory bladder occupying large portion of posterior body (Fig. 1B).

C. formosanus metacercariae (n = 30) were elliptical, 173-233 (208) × 135-178 (164) μm in size, had 32 circumoral spines around oral sucker arranged in 2 rows, and a X-shaped excretory bladder occupying greater portion of posterior body (Fig. 2A).

Metagonimus sp. metacercariae (n = 30) were elliptical, 138-200 (154) × 120-183 (144) μm in size, had yellow brownish pigment granules scattering in body area of intestinal bifurcation, a ventral sucker deflectively located from median, and a V-shaped excretory bladder (Fig. 2B).

C. sinensis metacercariae (n = 10) were elliptical, 158-193 (182) × 153-183 (168) μm in size, had 2 nearly equal sized suckers, brownish pigment granules scattering in the body, and an O-shaped excretory bladder occupying greater part of posterior body (Fig. 3A).

E. perfoliatus metacercariae (n = 10) were elliptical, 95-110

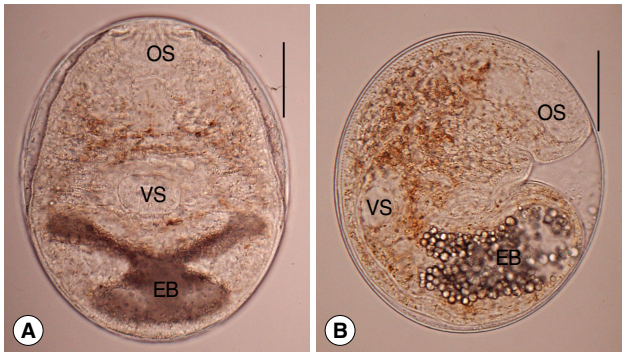

Fig. 2. (A) *Centrocestus formosanus* metacercaria detected in a freshwater fish, *Microphysogobio fukiensis*, from Mashan xian. It has 2 suckers, 32 circumoral spines around oral sucker (OS) arranged in 2 rows, and a X-shaped excretory bladder (EB) occupying greater portion of posterior body. Scale bar = 50 μm. (B) *Metagonimus yokogawai* metacercaria detected in a freshwater fish, *Zacco platypus*, from Yangshuo county. It is elliptical, has yellow brownish pigment granules scattering in body area of intestinal bifurcation, a ventral sucker (VS) deflectively located from median, and a V-shaped EB. Scale bar = 50 μm.

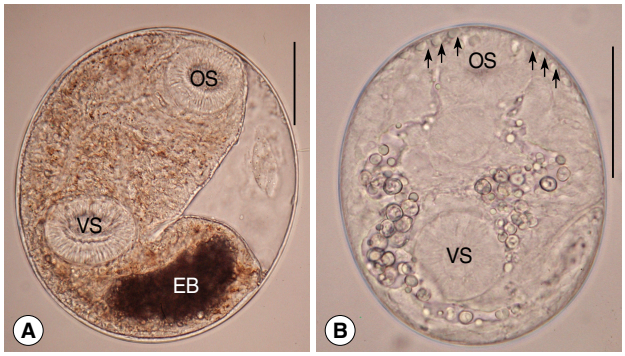

Fig. 3. (A) *Clonorchis sinensis* metacercaria detected in a freshwater fish, *Hemibarbus maculates*, from Yangshuo county. It is elliptical, has nearly equal sized two suckers, brownish pigment granules scattering in the body, and an O-shaped excretory bladder occupying greater part of posterior body. Scale bar = 50 μm. (B) *Echinochasmus perfoliatus* metacercaria detected in a freshwater fish, *Zacco platypus*, from Yangshuo county. It is elliptical, has a transparent and double layered cyst wall, 24 dorsally interrupted collar spines (arrow marks), and a ventral sucker (VS) lying median on posterior 1/3 of body and as large as oral sucker (OS). Scale bar = 50 μm.

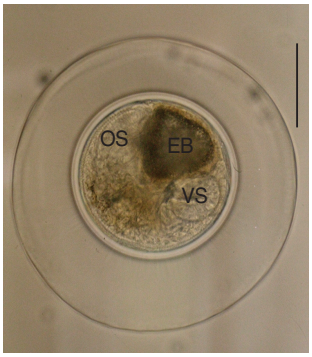

Fig. 4. *Metorchis taiwanensis* metacercaria detected in a freshwater fish, *Ctenopharyngodon idellus*, from Binyangxian. It is globular, has a double layered cyst wall (transparent and very thick outer layer and inner layer), nearly equal sized 2 suckers, and an O-shaped excretory bladder (EB). Scale bar = 50 μm.

(105) × 75-88 (85) μm in size, had a transparent and double layered cyst wall, 24 dorsally interrupted collar spines, and a ventral sucker lying median on posterior 1/3 of body and as large as oral sucker (Fig. 3B).

M. taiwanensis metacercariae (n = 10) were globular, 163-170 (166) × 150-170 (162) μm in size, had a double layered cyst wall (transparent and very thick outer layer: 43-52 μm and inner layer: 5.0-7.5 μm in thickness), 2 nearly equal sized suckers, and an O-shaped excretory bladder (Fig. 4).

Morphological characters of *Metagonimus yokogawai* adults (n = 20)

Adults of *M. yokogawai* were recovered from experimentally infected dogs and cats with metacercariae collected from fish from Yangshou county. They were 0.882 × 0.437 mm in average size, had the larger ventral sucker (av. 90 × 66 μm), deviated to right side from median, than oral sucker (av. 51 × 62), muscular pharynx (av. 37 × 32), moderately long esophagus (av. 88) and ceca. The round or elliptical ovary (av. 70 × 77) was located in median portion of body, and 2 adjacent testes (right: av. 139 × 107; left av. 127 × 98) were obliquely situated near the posterior end of body. The uterine loop passed through anterior border of both testis, and the uterine eggs were elliptical, yellow in color, 24.0-26.8 (av. 25.4) long and 15.0-17.5 (av. 15.6) wide (Fig. 5).

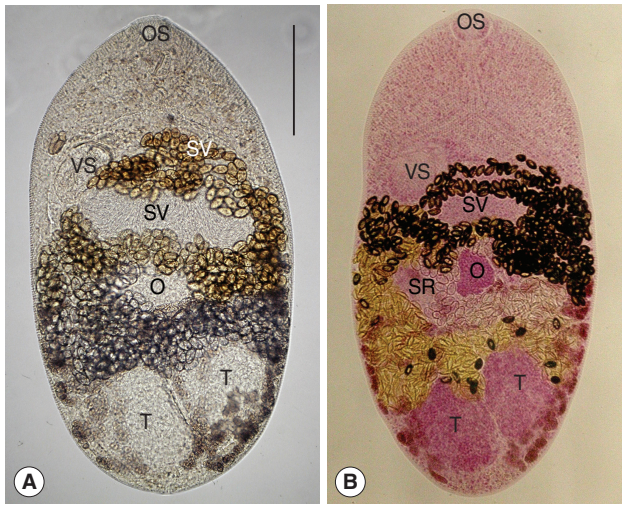

Fig. 5. Adults of *Metagonimus yokogawai* (A: unstained; B: Semichon's acetocarmine stained) were recovered from an experimental dog infected with metacercariae in fish from Yangshou county. They had a rightly deviated ventral sucker (VC), which is larger than the oral sucker (OS), muscular pharynx, moderately long esophagus and ceca, transversely long seminal vesicle (SV), round or elliptical ovary (O) and seminal receptacle (SR), and 2 obliquely adjacent testes (T). The uterine loop is passing through the anterior border of both testes. Scale bar = 200 μm.

DISCUSSION

World Health Organization (WHO) estimated that more than 18 million people are infected with fishborne trematodes (FBT), and more than half of billion people are at risk of FBT infections around the world [1,13]. FBT infections are classified as zoonoses because they are viewed as animal diseases transmitted to human. Some infections appear to be rare while others are common and cause serious disease. It has been known that liver flukes, *C. sinensis* and *Opisthorchis* spp., and some groups of intestinal flukes are representative FBT, and they are mainly infected by eating raw meat of freshwater and estuarine fish in low- and middle-income countries. On the other hand, it has been revealed that so many peoples are infected with FBT including *C. sinensis* in GZAR, China by the work of "Korea-China Collaborative Project of Control Strategies for Helminthiasis" [11]. However, it has not been obvious that what kinds of fish play a role of second intermediate host of FBT in this area. Therefore, present study was performed to investigate the infection status of FBT metacercariae in freshwater fish from GZAR, China.

Through this study, it was confirmed that some species of FBT metacercariae are prevalent in freshwater fish from GZAR, China. Especially, four species of intestinal flukes, *H. taichui, H. pumilio, M. yokogawai* and *C. formosanus,* were prevalent in fish hosts while *C. sinensis* metacercariae were detected from only 3 species of fish. Besides aforementioned 5 species, *E. perfoliatus* and *M. taiwanensis* metacercariae were also found in this study. Moreover, it was proved for the first time that 4 trematode species, *H. pumilio, M. yokogawai, E. perfoliatus* and *M. taiwanensis,* are distributed in GZAR, China [10].

About 70 species belonging to the 14 families, i.e., Hetero-

phyidae, Echinostomatidae, Plagiorchiidae, Lecithodendriidae, Neodiplostomidae, Nanophyetidae, Paramphistomatidae, Cathaemaciidae, Fasciolidae, Gastrodiscidae, Gymnophallidae, Microphallidae, Strigeidae and Brachylaimidae, have been found in the intestinal tract of humans around the world. Among members in these families, it has been known that Heterophyidae and Echinostomatidae are the major groups [14]. In China, it has been revealed that *Fasciolopsis buski* is most important intestinal fluke by the prevalence and morbidity, and 8 species of Heterophyidae *(M. yokogawai, M. takahashii, Stellantchasmus falcatus, C. formosanus, H. pumilio, H. taichui* and *Haplorchis yokogawai)* and 5 species of Echinostomatidae *(Echinostoma angustitestis, Echinochasmus japonicus, E. perfoliatus, Echinochasmus liliputanus* and *Echinochasmus fujianensis)* are reported as the intestinal FBT [10].

H. taichui metacercariae have been recorded in fish from several Asian countries, i.e. India, Thailand, the Philippines, Lao PDR and China [10, 15-20]. 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, *Barbonymus gonionotus, Puntius brevis,* and *Puntius orphoides,* in Thailand, 11 fish species, *Hampala dispar, Hampala macrolepidota, B. gonionotus, Mystacoleucus maginatus, P. brevis, Puntioplites falcifer, Cyclocheilchthys armatus, Cyclocheilchthys repasson, Onychostoma elongatum, Labiobarbus leptocheila,* and *Cirrhnus molitorella,* in Lao PDR, and 7 fish species, *Carassius auratus, Cyprinus carpio, Ctenopharyngodon idella, Pseudorasbora parva, Gambusia affinis, Rhodeus ocellatus* and *Puntius binotatus,* in China [10,15-20]. In the present study, *H. taichui* metacercariae were detected in 15 fish species including 3 susceptible ones such as, *H. leucisculus, C. carpio* and *T. houdemeri.* Moreover, it was confirmed that 14 fish species, *H. molitrix, H. maculatus, T. houdemeri, P. dispar, O. bidens, S. argentatus, A. rivularis, P. semifasciolatus, H. leucisculus, C. recurviceps, S. dabryi, M. lineata* and *C. dabryi,* are recorded as new second intermediate hosts of *H. taichui* in the literature. On the other hand, it had been known that *H. taichui* is distributed in only Taiwan, China. However, *H. taichui* adults were collected from residents of GZAR, China after praziquantel treatment and magnesium salt purgation by the work of "Korea-China Collaborative Project of Control Strategies for Helminthiasis" [11].

Distribution of *H. pumilio* has been recorded in Guangdong and Fujian Provinces and Taiwan, China [9]. However, it has been confirmed by this study that *H. pumilio* is distributed in GZAR also. Seventeen fish species except *P. parva, i.e., C. auratus, A. tonkinensis, H. maculates, H. molitrix, H. leucisculus, C. idella, T. houdemeri, M. fukiensis, P. dispar, O. bidens, S. argentatus, M. lineata, C. carpio, P. semifasciolatus, S. dabryi, C. recurviceps* and *C. dabryi* are listed as the new second intermediate hosts of this fluke in China. On the other hand, it has been reported that the prevalence of *H. pumilio* is 0.5% in Zhangzhou of Fujian Province by the special processing way of fish dish [10]. Many human cases by this fluke may be present in GZAR also because of many residents like to eat raw fish and they can easily infect with FBT.

C. formosanus has been reported in Guangdong, Fujian, GZAR, and Taiwan, and 8 fish species, *Anabas testudineus, Channa formosana, Clarias fuscus, Macropodus opercularis, G. affinis, C. autatus, C. carpio, C. idella,* have been recorded as the second intermediate hosts of this fluke in China [10]. In the present study, 10 fish species including susceptible *M. fukiensis* were revealed to be the second intermediate host or the source of infection of *C. formosanus.* Among them, 9 fish species, *M. fukiensis, A. tonkinensis, S. argentatus, H. molitrix, A. rivularis, H. leucisculus, M. lineate, S. dabryi* and *P. parva*, were added as new second intermediate hosts for *C. formosanus* in China. Recently, metacercariae of this fluke were detected in some fish species, i.e., *Esomus longimanus, P. brevis, C. repasson, O. hasseltii,* and *C. molitorella,* from Lao PDR [15,20,21].

In China, 2 *Metagonimus* species, *M. yokogawai* and *M. takahashii,* have been reported. *M. yokogawai* has been distributed in Taiwan, Guangdong, Anhui, Hubei and Zhejiang, while *M. takahashii* has been reported in Anhui Province only. Twelve fish species, *Abramis brama, Abramis ballerus (= Ballerus ballerus), Aspius aspius, Blicca bjoerkna, Carassius carassius, Chondrostoma nasus, Hemibarbus labeo, Leuciscus idus, Pelecus cultratus, Pseudobagrus fulvidraco (= Pelteobagrus fulvidraco), T. hakonensis* and *Zacco platypus*, have been recorded as the second intermediate hosts of *M. yokogawai* in China [9]. In the present study, *M. yokogawai* metacercariae were detected in 18 fish species from Yangshou county, and only one fish species, *Hemibarbus maculatus,* from a market in Nanning, GZAR. Among them,16 fish species, *Acrossocheilus hemispinus, Discogobio tetrabarbatus, H. maculates, Puntius semifasciolatus, Sinibrama macrops, Acheilognathus tonkinensis, Abbottina rivularis, Pseudogobio esocinus, C. auratus, Coreoperca whiteheadi, Opsariichthys bidens, Squalidus wolterstorffi, C. carpio, Ancherythroculter lini, Pseudohemiculter dispar and Hemiculter leucisculus,* were newly recorded as the second intermediate host of *M. yokogawai* in China.

Morphologically, adults of *Metagonimus* sp. recovered from experimentally infected animals with metacercariae, which col-

lected in fish from Yangshou county, were identical with those of *M. yokogawai* except the size of eggs. They have the larger ventral sucker, deviated to right side, than oral sucker, and the uterine loop passes through anterior border of both testes, whereas it courses between both testes in *M. takahashii* and *M. miyatai*. Their eggs, 25.4 × 15.6 μm in average size, were somewhat smaller than those of 3 *Metagonimus* species in Korea and Japan [22]. Biologically, the host specificity of this fluke relatively low when it is compared with those of 3 *Metagonimus* species, i.e. *M. yokogawai*, *M. takahashii* and *M. miyatai*, distributed in Korea [22]. As their fish intermediate hosts, *Plecoglossus altivelis*, *Tribolodon hakonensis*, *T. taczanowskii* and *Lateolabrax japonicus* for *M. yokogawai*, *C. carpio* and *C. auratus* for *M. takahashii*, *Zacco platypus* and *Z. temmincki* for *M. miyatai* have been reported in Korea [23,24].

C. sinensis infections are distributed in 22 out of 30 PRM (Province/Autonomous Region/Municipality) in China. In a nationwide survey, the prevalence was 0.4% among about 1.5 million people examined. Especially, the prevalence in Guangxi (1.39 %), with those in Guangdong (2.09%), Anhui (1.37%) and Heilongjiang (1.19%), was much higher than those of the other regions. Based on data of nationwide survey, the number of clonorchiasis patients in China may be about 6 million [7-9]. On the other hand, as the second intermediate hosts of *C. sinensis*, 102 fish species in 59 genera 15 families have been reported in China including Taiwan [9]. However, metacercariae of *C. sinensis* were detected in only 3 fish species, *H. maculatus*, *C. dabryi* and *H. leucisculus*, in this study. Moreover, their infection rates and intensities were very low.

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