

Foodborne Trematode Metacercariae in Fish from Northern Vietnam and Their Adults Recovered from Experimental Hamsters

Jong-Yil Chai¹, Nguyen Van De² and Woon-Mok Sohn^{3,*}

¹Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine, and Institute of Endemic Diseases, Seoul National University Medical Research Center, Seoul 110-799, Korea; ²Department of Parasitology, Hanoi Medical University, Hanoi, Vietnam; ³Department of Parasitology and Institute of Health Sciences, Gyeongsang National University School of Medicine, Jinju 660-751, Korea

Abstract: The prevalence of foodborne trematode (FBT) metacercariae was investigated in fish from 2 localities of northern Vietnam in 2004–2005. Freshwater fish (9 species) were collected from local markets in Hanoi City (n=76) and Nam Dinh Province (n=79), and were examined for FBT metacercariae using the artificial digestion technique. Adult flukes were obtained from hamsters experimentally infected with the metacercariae at day 8 post-infection. Three (*Haplorchis pumilio*, *Centrocestus formosanus*, and *Procerovum varium*) and 6 (*Haplorchis taichui*, *H. pumilio*, *C. formosanus*, *P. varium*, *Stellantchasmus falcatus*, and *Heterophyopsis continua*) species of FBT metacercariae were detected in the 2 regions, respectively. Overall, among the positive fish species, *H. pumilio* metacercariae were detected in 104 (80.0%) of 130 fish examined (metacercarial density per infected fish; 64.2). *C. formosanus* metacercariae were found in 37 (40.2%) of 92 fish (metacercarial density; 14.7). *P. varium* metacercariae were detected in 19 (63.3%) of 30 fish (*Anabas testudineus* and *Mugil cephalus*) (metacercarial density; 247.7). *S. falcatus* metacercariae were found in all 10 *M. cephalus* examined (metacercarial density; 84.4). *H. continua* metacercariae (2 in number) were detected in 1 fish of *Coilia lindmani*. Morphologic characteristics of the FBT metacercariae and their experimentally obtained adults were described. The results have demonstrated that various FBT species are prevalent in northern parts of Vietnam.

Key words: *Haplorchis taichui*, *Haplorchis pumilio*, *Centrocestus formosanus*, *Procerovum varium*, *Stellantchasmus falcatus*, *Heterophyopsis continua*, foodborne trematode, metacercaria, fish, Vietnam

INTRODUCTION

Foodborne trematode (FBT) infections are an important public health concern in various Asian countries, including Lao PDR, Vietnam, Cambodia, Thailand, the Philippines, Taiwan, China, and the Republic of Korea. Human FBT infections are caused by habitual consumption of raw fish containing infective larvae (metacercariae) [1-3]. Studies on FBT metacercarial infections have revealed that some species of freshwater and brackish water fish play important roles as the source of human infections in endemic areas [4-7]. It is known that FBT are not only pathogenic to the human host provoking remarkable morbidities but also harmful to fish hosts causing a seri-

ous economic loss in the aquaculture fish industry [1].

Surveys on metacercarial infection in second intermediate hosts, in combination with surveys on adult fluke infections in humans, are essential to understand the epidemiology and host-parasite relationships of FBT infections in particular geographical areas. However, fecal examinations are not usually suitable to determine the exact infection status in humans, since the specific identification of eggs is very difficult in cases of mixed infections with small-sized trematode eggs, in particular, the liver fluke, heterophyids, gymnophallids, and lecithodendriid flukes [8-10]. Therefore, investigation of metacercarial infections in second intermediate hosts available in the area around can provide valuable information on the trematode epidemiology.

Hanoi City and Nam Dinh Province are located in the northern part of Vietnam. Through adult fluke recovery by Dung et al. [11], it has been known that many residents in Nam Dinh Province are infected with FBT, such as *Clonorchis sinensis*, heterophyids (*Haplorchis pumilio*, *Haplorchis taichui*, *Haplorchis yok-*

•Received 15 July 2012, revised 12 August 2012, accepted 29 August 2012.

*Corresponding author (wmsohn@gnu.ac.kr)

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ogawai, and *Stellantchasmus falcatus*), and *Fasciolopsis buski* [11]. After that survey, metacercarial infections were investigated in fish intermediate hosts caught in several local areas in Vietnam [12-17]. In particular, Phan et al. [17] surveyed on FBT metacercarial infection in freshwater fish from small-scale farms (family-based household fish farm) in Nam Dinh Province. However, those studies did not provide quantitative details of FBT metacercarial infections in fish hosts, mainly focusing on qualitative aspects of commercially important cultured fish species. Therefore, in the present study, we intended to reveal the infection status (both qualitative and quantitative aspects) of FBT metacercariae in wild fish caught from 2 localities of northern Vietnam (Hanoi and Nam Dinh Province). In addition, we described the morphologic characteristics of FBT metacercariae and their adults obtained from experimentally infected hamsters.

MATERIALS AND METHODS

In Hanoi and Nam Dinh Province (Fig. 1), 76 and 79 fish (9



Fig. 1. Surveyed areas (■) (Hanoi City and Nam Dinh Province), located in northern Vietnam.

species each), respectively, were collected from local markets in November 2004 to April 2005. All collected fish were transferred with ice to the laboratory of Department of Parasitology, Gyeongsang National University School of Medicine, Jinju, Korea. Their length and weight were measured and the species were identified with the aid of FishBase site in internet (Table 1) [18]. Individual fish was 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 hr. The digested material was filtered through a 1 × 1 mm mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined using a stereomicroscope. The metacercariae were classified by their general features and were identified at the species level based on detailed morphologies and demensions using a light microscope. Identified metacercariae were experimentally infected to hamsters to obtain adult worms. At day 8 after infection, the hamsters were killed by cervical dislocation, and their small intestines were isolated and longitudinally opened with a scissors in a beaker containing 0.85% saline. Adult flukes were recovered from the sediment of the intestinal contents which were diluted and washed with 0.85% saline. Recovered worms were fixed with 10% formalin under a cover glass pressure, stained with Semichon's acetocarmine, and observed using a light microscope equipped with a micrometer.

RESULTS

FBT metacercariae in fish from Hanoi

In fish from Hanoi, 3 species of metacercariae, *H. pumilio*, *Centrocestus formosanus*, and *Procerovum varium* were recovered. *H. pumilio* metacercariae were detected in 7 of 9 fish species examined. Among them, *Ctenopharyngodon idella* was most heavily infected (Table 2). *C. formosanus* metacercariae were found in 4 fish species, and *Anabas testudineus* revealed a relatively high infection rate and high metacercarial density (Table 3). *P. varium* metacercariae were detected in only 1 fish species, *A. testudineus*. The infection rate of this fish with *P. varium* metacercariae was 100%, and the average metacercarial density was 466 per fish (Table 4).

FBT metacercariae in fish from Nam Dinh Province

In fish from Nam Dinh Province, 6 species of metacercariae, *H. taichui*, *H. pumilio*, *C. formosanus*, *P. varium*, *S. falcatus*, and *Heterophyopsis continua*, were recovered. *H. taichui* metacercari-

Table 1. Fish collected from local markets in Hanoi City and Nam Dinh Province, Vietnam

Species of fish	No. of fish examined	Length (cm)		Weight (g)	
		Range	Average	Range	Average
Fish from Hanoi ^a					
<i>Cirrhinus molitorella</i>	18	6.0-8.2	7.1	3.4-10.8	7.4
<i>Hypophthalmichthys molitrix</i>	10	6.0-6.8	6.4	2.4-3.9	3.3
<i>Carassius auratus</i>	10	5.0-7.0	6.1	3.3-10.6	6.5
<i>Anabas testudineus</i>	10	5.0-6.6	5.8	4.1-8.9	6.5
<i>Albulichthys albuloides</i>	10	5.2-7.5	6.1	2.6-7.9	4.6
<i>Parabramis pekinensis</i>	7	7.0-8.6	7.6	7.0-14.2	9.0
<i>Puntius semifasciolatus</i>	5	4.0-4.7	4.3	1.5-3.4	2.2
<i>Barbodes balleroides</i>	3	4.5-5.2	4.7	2.1-3.6	2.7
<i>Ctenopharyngodon idella</i>	3	7.3-11.4	9.2	6.2-25.3	14.9
Fish from Nam Dinh ^b					
<i>Carassius auratus</i>	21	4.6-8.2	5.8	2.6-12.6	5.4
<i>Ctenopharyngodon idella</i>	11	5.7-10.0	7.8	4.0-16.2	9.1
<i>Mugil cephalus</i>	10	7.4-11.0	9.4	7.8-19.3	14.0
<i>Anabas testudineus</i>	10	5.5-8.3	6.8	5.6-16.7	10.4
<i>Puntius semifasciolatus</i>	10	4.5-5.8	5.0	2.7-5.5	3.7
<i>Rasbora aurotaenia</i>	5	8.9-9.8	9.5	6.0-11.3	9.2
<i>Coilia lindmani</i>	5	16.2-17.6	17.0	21.0-31.0	25.5
<i>Trichogaster trichopterus</i>	5	6.4-7.1	6.8	9.5-12.4	10.9
<i>Acheilognathus barbatulus</i>	2	4.8-5.9	5.4	3.4-4.8	4.1

^a76 fish and ^b79 fish in total (9 species each) were examined.

Table 2. *Haplorchis pumilio* metacercarial infection in fish from local markets in Hanoi City and Nam Dinh Province, Vietnam

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Fish from Hanoi					
<i>Cirrhinus molitorella</i>	18	18 (100)	431	11-50	23.9
<i>Hypophthalmichthys molitrix</i>	10	9 (90.0)	92	3-22	10.2
<i>Carassius auratus</i>	10	3 (30.0)	5	1-2	1.7
<i>Albulichthys albuloides</i>	10	5 (50.0)	10	1-6	2.0
<i>Parabramis pekinensis</i>	7	6 (85.7)	46	2-14	7.7
<i>Ctenopharyngodon idella</i>	3	3 (100)	5,217	1,360-2,118	1,739
<i>Barbodes balleroides</i>	3	1 (33.3)	1	-	1.0
Subtotal	61	45 (73.8)	5,802	1-2,118	128.9
Fish from Nam Dinh					
<i>Carassius auratus</i>	21	20 (95.2)	188	1-26	9.4
<i>Anabas testudineus</i>	10	10 (100)	465	1-218	46.5
<i>Ctenopharyngodon idella</i>	11	9 (81.8)	191	1-43	21.2
<i>Puntius semifasciolatus</i>	10	8 (80.0)	27	1-8	3.4
<i>Rasbora aurotaenia</i>	5	4 (80.0)	14	2-8	3.5
<i>Coilia lindmani</i>	5	3 (60.0)	11	1-9	3.7
<i>Trichogaster trichopterus</i>	5	4 (80.0)	17	3-6	4.3
<i>Acheilognathus barbatulus</i>	2	1 (50.0)	35	-	35.0
Subtotal	69	59 (85.5)	878	1-218	14.9
Total	130	104 (80.0)	6,680	1-2,118	64.2

ae (6 in total number) were detected in 3 (60%) of 5 *Rasbora aurotaenia* fish. *H. pumilio* metacercariae were detected in 8 of 9 fish species examined. Among them, *A. testudineus* revealed relatively higher prevalence (Table 2). *C. formosanus* metacer-

Table 3. *Centrocestus formosanus* metacercarial infection in fish from local markets in Hanoi City and Nam Dinh Province, Vietnam

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Fish from Hanoi					
<i>Cirrhinus molitorella</i>	18	9 (50.0)	36	1-9	4.0
<i>Anabas testudineus</i>	10	9 (90.0)	403	7-194	44.8
<i>Parabramis pekinensis</i>	7	7 (100)	69	1-22	9.9
<i>Barbodes ballerooides</i>	3	1 (33.3)	4	-	4.0
Subtotal	38	26 (68.4)	512	1-194	19.7
Fish from Nam Dinh					
<i>Carassius auratus</i>	21	1 (4.8)	1	-	1.0
<i>Anabas testudineus</i>	10	4 (40.0)	9	1-4	2.3
<i>Ctenopharyngodon idella</i>	11	4 (36.4)	20	1-10	5.0
<i>Puntius semifasciolatus</i>	10	1 (10.0)	1	-	1.0
<i>Acheilognathus barbatulus</i>	2	1 (50.0)	2	-	2.0
Subtotal	54	11 (20.4)	33	1-10	3.0
Total	92	37 (40.2)	545	1-194	14.7

Table 4. *Procerovum varium* metacercarial infection in fish from local markets in Hanoi City and Nam Dinh Province, Vietnam

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Fish from Hanoi					
<i>Anabas testudineus</i>	10	10 (100)	4,655	25-1,928	465.5
Fish from Nam Dinh					
<i>Anabas testudineus</i>	10	3 (30.0)	37	5-21	13.5
<i>Mugil cephalus</i>	10	6 (60.0)	14	1-5	2.3
Subtotal	20	9 (45.0)	51	1-21	5.7
Total	30	19 (63.3)	4,706	1-1,928	247.7

cercariae were recovered in 5 fish species, and their infection rate and densities were relatively low (Table 3). *P. varium* metacercariae were detected in 2 fish species (climbing perch *A. testudineus* and striped mullet *Mugil cephalus*). *S. falcatus* metacercariae were detected in all 10 *M. cephalus*, and the average metacercarial density was 84.4. Only 2 *H. continua* metacercariae were recovered in a Lindman's grenadier anchovy, *Coilia lindmani*.

Morphology of metacercariae (All measurement unit is μm)

H. taichui metacercariae ($n=5$) were elliptical, 190-220 (av. 205) \times 160-190 (175) in size, had a baseball glove-shaped ventrogenital sac with 11-18 rodlets and an O-shaped excretory bladder occupying the large portion of the posterior body. These metacercariae were identical with those from Laotian and Chinese fish [5,6].

H. pumilio metacercariae ($n=20$) were elliptical, 160-195

(179) \times 145-175 (159) in size, had 36-42 deer horn-like minute spines arranged in 1-2 rows around the ventrogenital complex, and an O-shaped excretory bladder occupying the large portion of the posterior body. They were identical with those from Chinese fish [6].

C. formosanus metacercariae ($n=20$) were elliptical, 155-190 (169) \times 123-150 (138) in size, had 32 circumoral spines around the oral sucker arranged in 2 rows, and a X-shaped excretory bladder occupying the greater portion of the posterior body. They were identical with those from Laotian fish [5].

P. varium metacercariae ($n=20$) were elliptical, 165-208 (187) \times 115-163 (147) in size, had yellowish-brown pigment granules scattering in the area near the intestinal bifurcation, a pair of eyespots lateral to the pharynx, a submedian ventral sucker, a thick-walled bulb-like expulser, and a D-shaped (half moon-shaped) excretory bladder with grouped granules (Fig. 2A).

S. falcatus metacercariae ($n=10$) were elliptical, 255-330 (297) \times 225-250 (232) in size, had yellowish-brown pigment granules scattered all over the body, a submedian ventral sucker, a thick-walled bulb-like expulser, and a V-shaped excretory bladder (Fig. 2B).

H. continua metacercariae ($n=2$) were nearly round with relatively thick cyst wall, 440-475 (458) \times 435-470 (453) in size, had brownish pigment scattered all over the body, a ventral sucker located median, a genital sucker located just behind the ventral sucker, and a Y-shaped excretory bladder (Fig. 2C).

Morphology of adult flukes

H. taichui ($n=2$; Fig. 3A): Body small, pear-shaped, 580-730

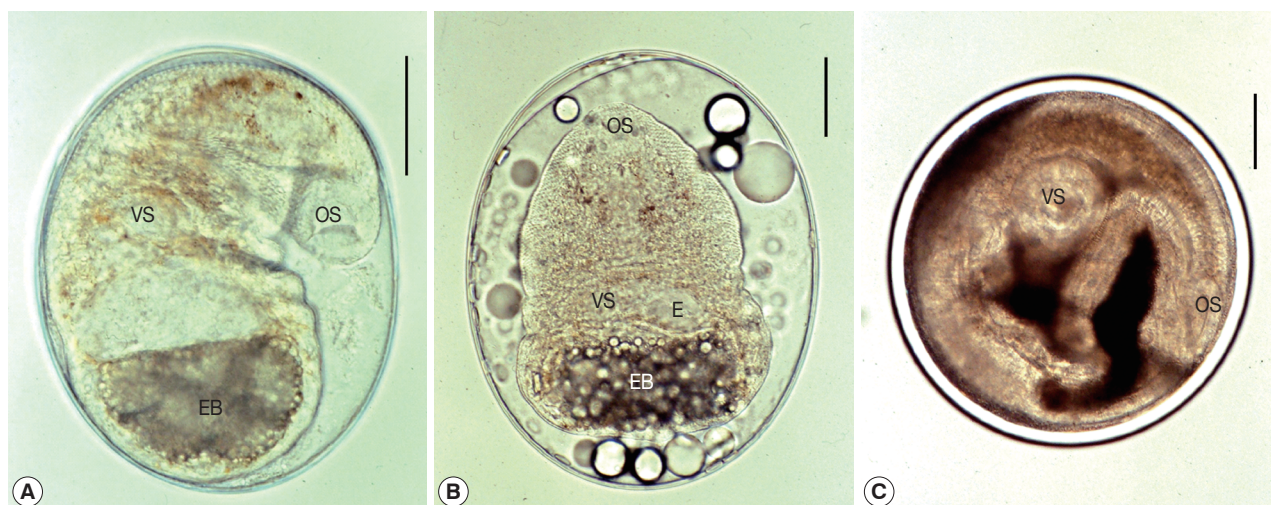


Fig. 2. FBT metacercariae detected in fish hosts from Hanoi and Nam Dinh Province, Vietnam. A) *P. varium* metacercaria; elliptical and $187 \times 147 \mu\text{m}$ in average size, having yellowish-brown pigment granules, a pair of eyespots, a muscular oral sucker (OS) and a submedian ventral sucker (VS), a thick-walled expulser, and a D-shaped excretory bladder (EB) with grouped granules. Scale bar = $50 \mu\text{m}$. B) *S. falcatus* metacercaria; elliptical and $297 \times 232 \mu\text{m}$ in average size, having yellowish-brown pigment granules, a muscular oral sucker (OS), and a submedian ventral sucker (VS), a thick-walled expulser (E), and a V-shaped excretory bladder (EB). Scale bar = $50 \mu\text{m}$. C) *H. continua* metacercaria; round with relatively thick cyst wall and $458 \times 453 \mu\text{m}$ in average size, having brownish pigments scattered all over the body, a muscular oral sucker (OS) and a ventral sucker (VS) located median, a genital sucker located just behind the ventral sucker, and a Y-shaped excretory bladder (EB). Scale bar = $100 \mu\text{m}$.

(av. 655) long and 300-310 (305) wide, with the greatest width at middle, the ovarian level. Oral sucker subterminal, 45 by 60 in size. Pharynx subglobular or elliptical, 35-38 (36) by 28-30 (29). Esophagus short, 75-95 (85) in length. Ventrogenital sac small with 11-18 rodlets, baseball glove-shaped, 88-100 (94) by 70-80 (75). Seminal vesicle saccular, bipartite, 113-125 (119) by 73-75 (74). Ovary spherical or subspherical, 85-88 (86) by 71-74 (73), dextral to midline. Seminal receptacle ellipsoidal, 80-88 (84) by 50-53 (51), lying at the right side of the ovary. Testis single, globular or subglobular, 160-193 (176) by 120-165 (143), lying in the posterior 1/4 of the body. Uterus with eggs occupying from the anterior 1/3 to the posterior end (most of the hind-body). Vitellaria follicular, distributing in post-ovarian fields. Eggs small, yellow, and 24-27 (26) by 12-14 (13).

H. pumilio (n = 15, Fig. 3B): Body small, pear-shaped, 415-550 (496) long and 195-245 (217) wide, with the greatest width at middle, the ovarian level. Oral sucker subterminal, 43-50 (46) by 50-58 (54). Pharynx subglobular or elliptical, 25-33 (29) by 20-30 (25). Esophagus short, 40-83 (57) in length. Ventrogenital sac small with 36-42 deer horn-like minute spines, 65-80 (74) by 50-75 (57). Seminal vesicle saccular, 28-100 (60) by 18-75 (45). Ovary spherical or subspherical, 55-70 (62) by 30-68 (52), slightly dextral to midline. Seminal receptacle elliptical, 38-85 (51) by 25-58 (38), lying at the right side of the

ovary. Testis single, globular or subglobular, 65-103 (82) by 75-105 (85), lying at the posterior 1/4 of the body. Uterus with eggs occupying from the anterior 1/3 to the posterior end (most of the hind-body). Vitellaria follicular, distributing in post-ovarian fields. Eggs small, yellow, and 28-31 (30) by 15-18 (16).

P. varium (n = 20, Fig. 3C): Body small, pear-shaped, 355-475 (434) long and 190-250 (223) wide, with the greatest width at posterior 1/3 of the body. Oral sucker subterminal, 35-43 (38) by 38-48 (42). Pharynx subglobular or elliptical, 23-30 (27) by 18-23 (20). Esophagus short, 33-75 (55) in length. Ventral sucker very small, 15-30 (25) by 18-33 (27), embedded in the ventrogenital sac. Expulsor long and thick-walled, 88-138 (115) by 25-35 (29). Ovary spherical or subspherical, 45-75 (59) by 38-70 (48), slightly dextral to midline. Seminal receptacle saccular, 45-83 (63) by 33-55 (44), lying at the right side of the testis. Testis single, globular or subglobular, 105-150 (127) by 100-180 (127), situated at the middle of the hind-body. Uterus with eggs occupying from the anterior 1/3 to the posterior end (most of the hind-body). Vitellaria follicular, distributing from the posterior border of the ovary to the posterior extremity. Eggs small, yellow, and 25-28 (26) by 11-15 (13).

S. falcatus (n = 15, Fig. 3D): Body small, 400-650 (481) long and 200-300 (239) wide. Oral sucker subterminal, 30-43 (38) by 45-55 (49). Pharynx subglobular, 23-30 (27) by 20-38 (24).

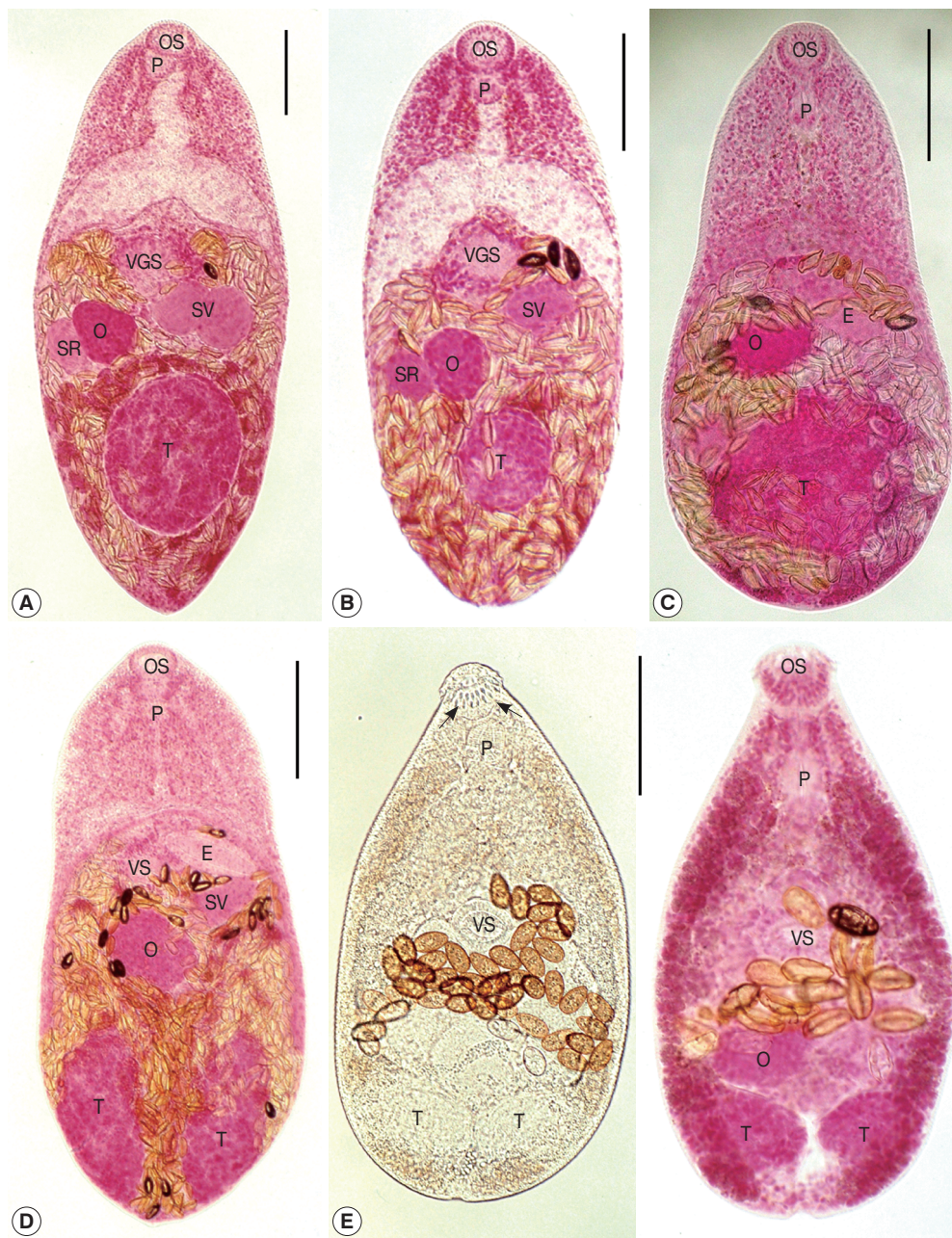


Fig. 3. Adult heterophyid flukes recovered from experimental hamsters at day 8 post-infection. All scale bars=100 μ m. A) *H. taichui* adult. Body small, 655 \times 305 μ m in average size, having a muscular oral sucker (OS) and pharynx (P), a ventrogenital sac (VGS) armed with 11-18 rodlets, a saccular seminal vesicle (SV) and seminal receptacle (SR), a spherical ovary (O), single globular testis (T), and follicular vitellaria distributed in post-ovarian fields. B) *H. pumilio* adult. Body small, 496 \times 217 μ m in average size, having a muscular oral sucker (OS) and pharynx (P), a ventrogenital sac (VGS) equipped with 36-42 deer horn-like minute spines, a saccular seminal vesicle (SV) and seminal receptacle (SR), a spherical ovary (O), single globular testis (T), and follicular vitellaria distributed in post-ovarian fields. C) *P. varium* adult. Body small, 434 \times 223 μ m in average size, having a muscular oral sucker (OS) and a pharynx (P), a small ventral sucker and a long and thick-walled expulsor (E), a spherical ovary (O), single globular testis (T), and follicular vitellaria distributed in post-ovarian fields. D) *S. falcatus* adult. Body small, 481 \times 239 μ m in average size, having a muscular oral sucker (OS) and pharynx (P), a small ventral sucker (VS) and a long and thick-walled expulsor (E), a spherical ovary (O), 2 globular testes (T), and follicular vitellaria distributed in post-ovarian fields. E) *C. formosanus* adult (left, unstained; right, acetocarmine-stained). Body very small, 367 \times 207 μ m in average size, having an oral sucker (OS) armed with 32 circumoral spines (arrow marks), a muscular pharynx (P), a well-developed ventral sucker (VS), a spherical ovary (O), 2 globular testes (T), and follicular vitellaria distributed along extracecal margins from the pharyngeal level to the posterior end.

Esophagus slender, 45-118 (72) in length. Ventral sucker small, 25-30 (27) by 28-30 (29). Expulsor long and thick-walled, 75-100 (87) by 30-38 (33). Seminal vesicle saccate, 63-70 (66) by 33-40 (37). Ovary spherical, 63-100 (79) by 43-75 (62). Testes paired, ovoid or globular, slightly oblique and widely separated; right 83-180 (111) by 45-93 (62); left 83-188 (108) by 30-88 (55). Uterus with eggs occupying from the anterior 1/3 to the posterior end (most of the hind-body). Vitellaria follicular, distributing in the post-ovarian fields. Eggs small, yellow, and 20-23 (21) by 11-13 (12).

C. formosanus (n=15, Fig. 3E): Body very small, 320-470 (367) long and 170-235 (207) wide. Oral sucker subterminal, 35-43 (40) by 38-50 (42), armed with about 32 circumoral spines. Prepharynx very short, 10-25 (17) long. Pharynx globular, 30-43 (35) by 20-28 (23). Esophagus very short. Ventral sucker round or elliptical, 33-43 (37) by 45-53 (51). Ovary elliptical, 50-75 (59) by 30-53 (39), dextral to midline. Seminal receptacle large and saccular, 50-75 (63) by 40-55 (49). Two testes ellipsoidal, side by side near the posterior end; right 60-88 (72) by 33-50 (41), left 55-88 (66) by 30-43 (36). Vitellaria follicular, distributing along the extracecal margins from the pharyngeal level to the posterior end. Eggs small, yellow, and 29-33 (30) by 15-18 (17).

DISCUSSION

The 6 species of FBT metacercariae (*H. taichui*, *H. pumilio*, *C. formosanus*, *P. varium*, *S. falcatus*, and *H. continua*) detected in this study were all minute intestinal flukes and members of the Heterophyidae. Among them, *H. pumilio* was the dominant species. It is of note that Dung et al. [11] reported a high prevalence (64.9%) of small trematode eggs and also high prevalence for soil-transmitted helminths among residents of 2 communes in Nam Dinh Province, northern Vietnam, in April 2005. They recovered adult flukes of 6 trematode species (*Clonorchis sinensis*, *H. pumilio*, *H. taichui*, *H. yokogawai*, *S. falcatus*, and *Fasciolopsis buski*) from 33 peoples who revealed over 1,000 eggs per gram of feces (EPG) for small trematode eggs after praziquantel treatment and purgation. Among the 6 trematode species, *H. pumilio* was recovered in all 33 (100%) residents and the worm load averaged 416 per person [11]. Accordingly, it is presumed that more than 8 species of FBT, including *H. pumilio*, may be distributed in the northern part of Vietnam, although we could not find *C. sinensis* and *H. yokogawai* metacercariae.

Recently, studies on FBT metacercarial infections in Vietnam have been performed popularly. Thu et al. [12] surveyed on metacercarial infections in cultured catfish and snakeheads, and also in several wild fish species from An Giang Province, a major fish production area in the Mekong Delta of Vietnam in 2005-2006. Thien et al. [13] examined 13 major cultured fish species from Tien Giang Province and Can Tho City, located in central areas of the Mekong Delta of Vietnam, in 2005-2006. Hop et al. [14] examined FBT metacercarial infections in wastewater-fed aquaculture fish in northern Vietnam [14]. Chi et al. [15] performed a cross-sectional survey of FBT metacercariae in farmed fish in Nghe An Province, about 300 km south of Hanoi, in 2005 [15]. Vo et al. [16] surveyed 2 species of groupers (*Epinephelus coioides* and *Epinephelus bleekeri*), and mullet (*M. cephalus*) in a central coastal area of Vietnam (Khanh Hoa Province) in 2008 [16]. Phan et al. [17] surveyed on the infection status of FBT metacercariae in freshwater fish from small-scale farms (family-based household fish farm) in Nam Dinh Province in 2010 [17]. These studies focused mainly on metacercarial infections in commercially important fish species and in qualitative aspects. Studies on quantitative aspects of FBT metacercarial infections in each species of fish and thereby the suitability and susceptibility of fish hosts for each FBT species have not been available.

In the present study, *H. pumilio* metacercariae were detected in 104 (80.0%) of 130 fish examined, and the metacercarial density was 64.2 per fish infected. The metacercarial density was higher in fish from Hanoi (av. 129 metacercariae) than those from Nam Dinh Province (av. 15 metacercariae). The most heavily infected fish with *H. pumilio* metacercariae was the grass carp (*Ctenopharyngodon idella*) caught from Hanoi. The high susceptibility of this fish species was also shown in the study of Phan et al. [17] which was performed in Nam Dinh Province [17]. A similar finding was shown in Guangxi Zhuang Autonomous Region located in the southern part of China, slightly north of our surveyed area [6]. In this area of China, 18 fish species (*Carassius auratus*, *Acheilognathus tonkinensis*, *Hemibarbus maculatus*, *Hypophthalmichthys molitrix*, *Hemiculter leucisculus*, *C. idella*, *Toxabramis houdemeri*, *Microphysogobio fukiensis*, *Pseudohemiculter dispar*, *Opsariichthys bidens*, *Squalidus argentatus*, *Metzia lineata*, *Cyprinus carpio*, *Puntius semifasciolatus*, *Saurogobio dabryi*, *Culter recurviceps*, *Chanodichthys dabryi*, and *Pseudorasbora parva*) were listed as hosts for *H. pumilio* [6]. On the other hand, in Nam Dinh Province of Vietnam, Phan et al. [17] listed 17 fish species (*Labeo rohita*, *H. molitrix*, *Cirrhinus*

mrigala, *C. idella*, *C. auratus*, *Squaliobarbus curriculus*, *Piaractus brachypomum*, *C. carpio*, *Cirrhinus molitorella*, *Hypophthalmichthys nobilis*, *Barbonymus gonionotus*, *H. leucisculus*, *Anabas testudineus*, *Oreochromis niloticus*, *Clarias batrachus*, *Channa orientalis*, and *Notopterus notopterus*) for the host for *H. pumilio*. In the present study, 7 more fish species (*Acheilognathus barbatulus*, *Albulichthys albuloides*, *Barbodes balleroides*, *Coilia lindmani*, *Parabramis pekinensis*, *Rasbora aurotaenia*, and *Trichogaster trichopterus*) have been newly added as the host for *H. pumilio*.

C. formosanus metacercariae were detected in 8 fish species (*A. barbatulus*, *A. testudineus*, *B. balleroides*, *C. auratus*, *C. molitorella*, *C. idella*, *P. pekinensis*, and *P. semifasciolatus*) in the present study. Phan et al. [17] detected the same species of metacercariae in 10 fish species (*L. rohita*, *H. molitrix*, *C. mrigala*, *C. idella*, *S. curriculus*, *P. brachypomum*, *C. carpio*, *C. batrachus*, *C. orientalis*, and *A. testudineus*) in Nam Dinh Province [17]. In China, Sohn et al. [6] reported 10 fish species (*M. fukiensis*, *A. tonkinensis*, *S. argentatus*, *C. carpio*, *H. molitrix*, *A. rivularis*, *H. leucisculus*, *M. lineata*, *S. dabryi* and *P. parva*) as the fish hosts for *C. formosanus* [6]. Therefore, it appears that more than 24 fish species play the role of second intermediate hosts for *C. formosanus* in Vietnam and China.

It has been known that 2 species of liver flukes (*Clonorchis sinensis* and *Opisthorchis viverrini*) distribute in Vietnam. *C. sinensis* is distributed in the northern part, whereas *O. viverrini* is found in the southern part. However, the metacercariae of these liver flukes were only rarely detected in fish from Vietnam. In An Giang Province, a southern part, only 19 metacercariae of *O. viverrini* were detected in 1.9% of 108 fish examined [12]. Thien et al. [13] could not find any liver fluke metacercariae in all 13 important cultured fish species from Tien Giang Province and Can Tho City, in the Mekong Delta of Vietnam [13]. No liver fluke metacercariae were detected in a cross-sectional survey of FBT metacercariae in farmed fish from Nghe An Province [15]. Meanwhile, only a small number of *C. sinensis* metacercariae were found in 1 of 1,185 silver carps (*H. molitrix*) examined in small-scale farms in Nam Dinh Province. In the present study, we could not find any *C. sinensis* metacercariae in fish from the same study area. By contrast, Dung et al. [11] reported a high positive rate (51.5%) of *C. sinensis* adult worms (their worm load was not so high; 1-18 per individual) from 33 residents with over 1,000 EPG for small trematode eggs in Nam Dinh Province [11]. This discrepancy between the metacercarial infection in fish hosts and the adult fluke infections in humans should be clarified in the near future.

In the present study, *P. varium* metacercariae were detected in 2 fish species (*A. testudineus* and *M. cephalus*), although these metacercariae were previously recorded in 9 fish species (*L. rohita*, *H. molitrix*, *C. mrigala*, *C. idella*, *S. curriculus*, *P. brachypomum*, *C. batrachus*, *B. gonionotus*, and *A. testudineus*) in Nam Dinh Province [17]. Vo et al. [16] also detected *P. varium* metacercariae together with 2 other heterophyid metacercariae (*H. continua* and *P. summa*) in 2 species of groupers and mullet from Khanh Hoa Province in Vietnam. On the other hand, *S. falcatus* and *H. continua* metacercariae were found in *M. cephalus* and *C. lindmani*, respectively, in the present study.

Although morphologic characteristics of FBT metacercariae had previously been reported, we redescribed some of their characteristic features to provide a useful aid for epidemiologic studies in Southeast Asian countries. Among the FBT metacercariae detected in our study, 3 species (*H. taichui*, *H. pumilio*, and *C. formosanus*) were morphologically identical with those from China and Lao PDR [5,6]. On the other hand, the metacercariae of *P. varium* (187×147 µm) were smaller than those (210×180) reported in a freshwater fish (*Oryzias melastigma*) from Visakhapatnam, India [19]. However, our *P. varium* metacercariae were almost identical with those found in groupers from Nha Trang district in Khanh Hoa Province, Vietnam [16]. With regard to *S. falcatus* metacercariae, they were previously detected in cultured giant gourami (*Osphronemus gourami*) from Tien Giang Province and Can Tho City [13], and also in the common carp (*C. carpio*) and grass carp (*C. idella*) from Nghe An Province, Vietnam [15]. However, no morphologic descriptions were available on *S. falcatus* metacercariae in Vietnam. *H. continua* metacercariae (458×453 µm) detected in a *C. lindmani* fish in the present study were similar in shape with but larger than those (380 in diameter) recovered in mullets from Khanh Hoa Province [16]. In the Republic of Korea, it is agreed that *H. continua* metacercariae detected in various fish species (*Laterolabrax japonicus*, *Acanthogobius flavimanus*, *Clupanodon punctatus*, *Plecoglossus altivelis*, *Conger myriaster*, *Boleophthalmus pectinirostris*, and *Scartelaos* sp.) show wide size ranges by fish hosts examined [20].

Among the FBT species distributed in Vietnam, 2 species of liver flukes (*C. sinensis* and *O. viverrini*) are highly important in clinical and pathological aspects, although their metacercariae were not detected in this study. Being similar in general shape, the liver fluke metacercariae can be distinguished from those of heterophyid flukes by the presence of a large well-developed ventral sucker which is nearly equal in size with the oral suck-

er. The ventral suckers of *H. taichui*, *H. pumilio*, *C. formosanus*, *P. varium*, and *S. falcatus* are characteristically smaller than their oral suckers. Nevertheless, when these metacercariae were mixed together, it is not easy to distinguish them unless each metacercaria is subjected to a detailed observation under a light microscope.

The experimentally obtained adults of the 5 species of FBT were morphologically compatible with those previously reported. Dung et al. [11] only briefly mentioned on the adult worm morphologies of *H. pumilio*, *H. taichui*, *H. yokogawai*, and *S. falcatus* after recovery in residents of Nam Dinh Province, Vietnam. The size of *H. taichui* (756 × 421) and *H. pumilio* (632 × 291) adults obtained from residents were slightly larger than those from our study, whereas the adult of *S. falcatus* (468 × 298) from residents was smaller in size than our specimens [11].

ACKNOWLEDGMENTS

We thank the staff of the Fishborne Zoonotic Parasites in Vietnam (FIBOZOPA project), in particular, Dr. K. Darwin Murrell, Mr. Jesper Clausen, and Mr. Bui Thanh, for their kind cooperation. We also thank Jung-A Kim, Department of Parasitology, Gyeongsang National University School of Medicine, Jinju, Korea, for her help in fish examinations.

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