DNA FINGERPRINTS OF SOME HETEROPHYID TREMATODES FROM ADULT AND METACERCARIAL STAGES IN THAILAND

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Abstract. The DNA fingerprints of some trematodes in family Heterophyidae, *Stellantchasmus falcatus* and *Haplorchis taichui*, were investigated on adult and metacercarial stages. The other trematode, *Haplorchoides* sp was included. The molecular method, the high annealing temperature-random amplified polymorphic DNA (HAT-RAPD) was examined with eight arbitary primers (OPA-02, OPA-08, OPA-09, OPN-02, OPN-03, OPN-09, OPX-13, and OPH-19). The bands were shown between 160-2,865 bps. The specific bands of each trematode - *S. falcatus, H. taichui*, and *Haplorchoides* sp - were 49, 56, and 42 bands respectively; and three bands were found in all trematodes. Eight arbitary primers from this study could be used for detecting the DNA fingerprints and finding the specific primer of these trematodes in the further study.

INTRODUCTION

The heterophyid trematode is the common fluke, which infects humans and causes epidemic disease in North and Northeast Thailand. The adults are able to develop in the small intestine of birds and mammals, including humans (Jongsuksuntikul *et al*, 1992; Radomyos *et al*, 1998; Waikakul, 1998). The prevalence of metacercarial stage in fishes trends to increase, resulted in the increase of high prevalence of the adult stage in humans (Radomyos *et al*, 1998). Two heterophyid trematodes, *Stellantchasmus falcatus* and *Haplorchis taichui*, were reported highly prevalent in northern Thailand, both adult (Kliks and Tantachamrun, 1974; Tantachamrun and Kliks, 1978; Radomyos *et al*, 1998) and metacercarial stages (Saenphet *et al*, 2001; Sripalwit *et al*, 2003; Wongsawad *et al*, 2004). The identification of the egg stage was uncertain regarding the morphology (Radomyos *et al*, 1998; Chuboon and Wongsawad, 2003). Mixed infection of the metacercarial stage in fish with both *Haplorchis taichui* and *Haplorchoides* sp was often found (Namue *et al*, 1998; Kumchoo *et al*, 2003; Wongsawad *et al*, 2004). The molecular amplification method, high annealing temperature-random amplified polymorphic DNA (HAT-RAPD), was used effectively to identify heterophyid trematodes, such as *Stellantchasmus falcatus* (Sripalwit *et al*, 2003), and *Haplorchis taichui* (Wongsawad *et al*, 2007). The fingerprints of heterophyid trematodes using the PCR method were used to confirm the adult stage of *Metagonimus* spp (Yu *et al*, 1997). This study aimed to describe the fingerprints of adult and metacercarial stages of *Stellantchasmus falcatus* and *Haplorchis taichui*, as well as the out-group, *Haplorchoides* sp, which could be used to identify the species with certainty.

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MATERIALS AND METHODS

Parasitic materials

Metacercarial trematodes were collected from freshwater fishes as follows: *S. falcatus* from *Dermogenys falcatus*, and *H. taichui* and *Haplorchoides* sp from *Henicorhynchus siamensis* using 1% pepsin solution in a shaking waterbath at 370C for two hours. The adults of *S. falcatus* and *H. taichui* were prepared by oral force-feeding three-day old chicks (*Gallus gallus domesticus*) with metacercaria. Adults were collected and retained seven days postinfection from the small intestine using Baerman's apparatus. The adult of *Haplorchoides* sp was collected from the intestine of the *Hemibagrus filamentus*. All trematodes of both stages were identified to the species by observing the permanent slides under a light microscope. DNA fingerprints

The DNA fingerprints of some trematodes in the family Heterophyidae, *Stellantchasmus falcatus* and *Haplorchis taichui*, were investigated in the adult and metacercarial stages. The outgroup trematode, *Haplorchoides* sp, was also included. DNA was extracted using the DNeasy Tissue Kit (QIAGEN), 25 mg in a 1.5 ml microcentrifuge tube, and was eluted in 5 mM Tris-HCl, at pH 8.5. The molecular method by Anuntalabhochai *et al* (2000), the High Annealing Temperature-Random Amplified Polymorphic DNA (HAT-RAPD), was used with nine arbitary primers (OPA-02, OPA-08, OPA-09, OPN-02, OPN-03, OPN-09, OPX-13, OPP-15, and OPH-19). All arbitary primers were amplified DNA of all trematodes in both stages. The HAT-RAPD products were separated by 1.7 % agarose gel electrophoresis at 60 volts for 2 hours and 30 minutes, stained with 1 mg/ml of ethidium bromide for 10 minutes, and then destained in distilled water for 10 minutes (Wongsawad *et al*, 2007). The DNA bands were examined under UV transilluminator, and photographed by Kodak digital camera Gel LOGIC 100.

DNA analysis

The DNA patterns of banding of trematodes were compared with 100-bp DNA ladder plus (Fermantas) and 100-bp DNA ladder plus (BIO-RAD), and were investigated for the molecular weight using Kodak ID Image.

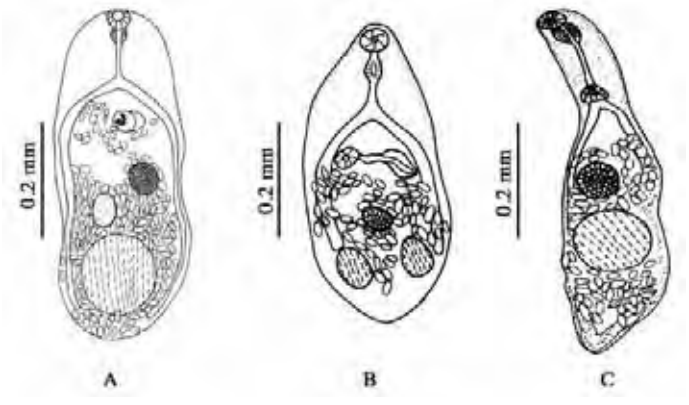

Fig 1- Trematodes: A: *Haplorchis taichui*, B: *Stellantchasmus falcatus*, C: *Haplorchoides* sp.

RESULTS

Morphological investigation

The species of trematodes were confirmed according to Pearson (1964), and Pearson and Ow-Yang (1982). The morphology and characteristics of adult worms were shown in Fig 1 (A-C). The entire body of trematodes was difficult to generally describe, but the unique characteristics of each species was observed. *S. falcatus* has a submedian ventrogenital sac with two dense minute spines, while *H. taichui* has fan-shaped acetabular spines, and *Haplorchoides* sp has long intestinal ceca at the posterior end of the body.

DNA fingerprints

Eight of the arbitary primers were the amplified DNA of all trematodes in both stages except OPP-15, which had not amplified the DNA of *S. falcatus*. The arbitary primers amplified the DNA fingerprint of each trematode in the same pattern of both stages. The results in terms of total banding number and different bands are presented in Table 1. The pattern of DNA fingerprints of both stages of the trematodes were distinguished as appear in Fig 2 (A-H). A total of 180 bands were examined as molecular weight by Kodak ID Image between 160-2,865 bps. Three bands were found in all the trematodes of the both stages by OPA-02, OPA-08, and OPX-13, with molecular weights of 580, 650, and 650 bps, respectively. The specific bands of each species were analyzed. The OPN-02 primer had the highest band number (36) and produced the highest specific band number (32). The specific bands for each trematode-*S. falcatus, H. taichui*, and *Haplorchoides* sp-were 49, 56, and 42 bands, respectively; and 3 bands were found in all trematodes.

DISCUSSION

The entire body of trematodes could be identified, with the unique characteristics of each species, but by the specialist parasitologist only. The reproductive system and unique

Table 1
The molecular weight of specific bands amplified by eight arbitary primers.

Primer	Bases sequence 5'--> 3'	Total DNA bands	Total specific bands	Molecular weight of the specific bands (bps)
OPA-02	TGCCGAGCTG	19	14	S- 1,270, 935, 875 HC- 1,300, 780, 750, 450, 190 HP - 1,330, 830, 725, 590, 130
OPA-08	GTGACGTAGG	16	14	S- 1,515, 990, 395 HC- 1,100, 970, 860, 555, 440 HP- 1,450, 1015, 875, 760, 460, 405
OPA-09	GGGTAACGCC	20	14	S- 880 , 805 , 604 , 430 , 340 , 250 HC- 960 HP- 1,080, 995, 640, 565, 407, 215
OPN-02	ACCAGGGGA	36	32	S- 1,540, 1440, 1,190, 1,110, 990, 860, 810, 782, 610, 525, 412, 365, 190 HC- 2,250, 1,465, 1,348, 1,230, 1,125, 960, 870, 815, 750, 650, 555, 435 HP- 1,088, 990, 780, 630, 405, 350, 216
OPN-03	TGCCGGCTTG	25	22	S- 1,900, 1,605, 1,290, 1,270, 1,140, 955, 510, 470 HC- 1,820, 1,410, 1,240, 1,195, 1,060, 780, 540 HP- 1,230, 820, 480, 390, 340, 245, 230
OPN-09	TGCCGGCTTG	22	21	S- 960, 900, 850, 545, 460, 370 HC- 1,380, 1,200, 990, 915, 830, 745, 560, 535, 515, 471, 395, 335 HP- 1,165, 1,005, 500
OPX-13	ACGGGAGCAA	16	12	S- 950, 670, 560, 300 HC- 1,100, 855, 760, 635, 370, 280 HP- 1,870, 390
OPH-19	CTGACCAGCC	26	20	S- 1,885, 1,235, 940, 735, 512, 250 HC- 2,865, 2,770, 2,420, 1,335, 1,145, 870, 760, 270 HP- 770, 665, 505, 487, 450, 345

S = *Stellantchasmus falcatus*; HC = *Haplorchis taichui*; HP = *Haplorchoides* sp.

characteristics organs appear distinctly in the adult stage. The mixed infections of trematodes are also difficult to identify (Namue *et al*, 1998; Wongsawad *et al*, 2004).

Eight arbitary primers from this study could be used to identify the trematode species in both adult and metacercarial stages. The protocol for detecting could be used in all species. However, adult *S. falcatus* and *H. taichui*, had to be prepared from the metacercarial stage from fish in order to confirm the species. The adult and metacercarial stages of *Haplorchoides* sp were collected from different hosts. The DNA patterns of both stages were confirmed as the same species that was introduced in the life cycle in the second intermediate host and definitive host. This method could be used accurately to detect the larval stage in the first intermediate host, freshwater snails, and the egg stage of adult worm in animals or humans. Previous

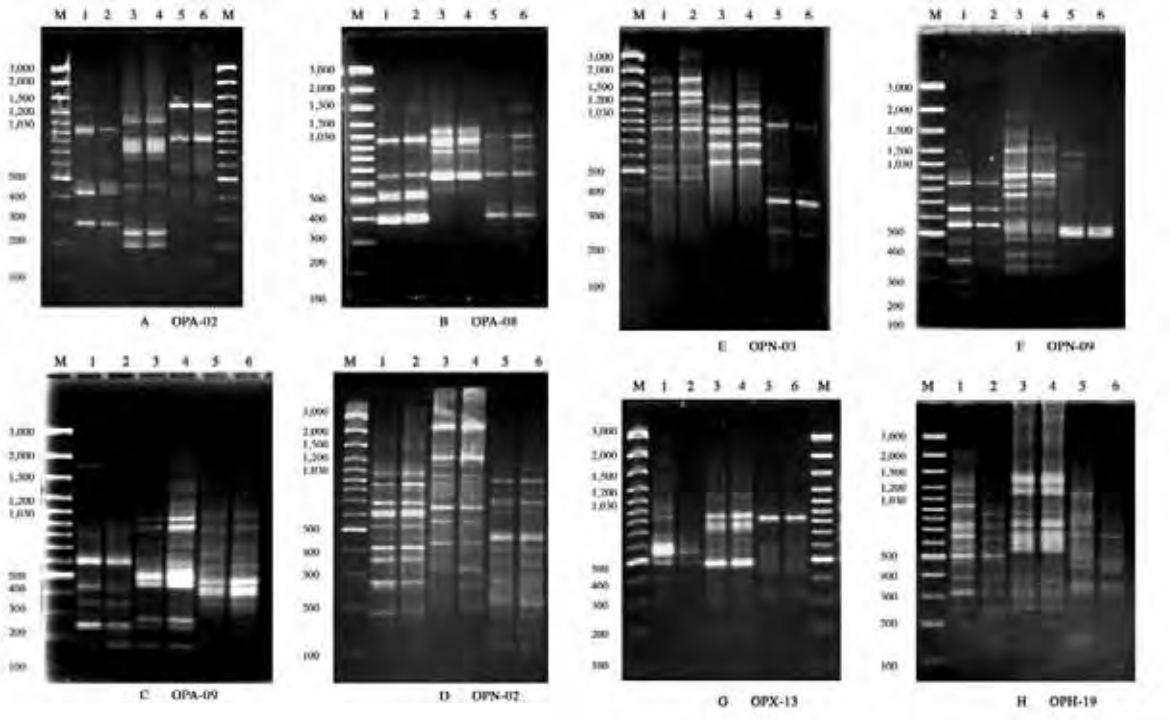

Fig 2- The fingerprints of trematode DNA by HAT-RAPD with primers: A: OPA02, B: OPA-08, C: OPA-09, D: OPN-02, E: OPN-03, F: OPN-09, G: OPX-13, H: OPH-19; (lane M: 100-bp ladder plus, lane 1: adult *S. falcatus*, lane 2: metacercaria *S. falcatus*, lane 3: adult *H. taichui*, lane 4: metacercaria *H. taichui*, lane 5: adult *Haplorchoides* sp, lane 6: metacercaria *Haplorchoides* sp).

reports have illustrated the similarity of the egg stages of heterophyid trematodes and liver fluke (Kliks and Tantachamrun, 1974; Radomyos *et al*, 1998; Kumchoo *et al*, 2003; Wongsawad et al, 2003). The DNA fingerprint of the heterophyid trematode, *Metagonimus* spp, was detected by Yu *et al* (1997) and Yang *et al* (2000), respectively. Recently, the HAT-RAPD method has been used to analyzed the DNA fingerprint of *Stellantchasmus falcatus* (Sripalwit *et al*, 2003), and the DNA quantities and qualities of some trematodes (Wongsawad *et al*, 2007). From this study, eight arbitary primers could be used to detect the DNA fingerprints of these trematodes and to find the specific primer of each trematode for further study.

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