

MOLECULAR IDENTIFICATION OF LARVAL TREMATODE IN INTERMEDIATE HOSTS FROM CHIANG MAI, THAILAND

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Abstract. Snail and fish intermediate hosts were collected from rice fields in 3 districts; Mueang, Mae Taeng and Mae Rim of Chiang Mai Province during April-July 2008. For identification of larval trematode infection, standard (cracked for snail and enzymatically digested for fish) and molecular methods were performed. The results showed that three types of cercariae were found, pleurolophocercus, cotylocercous, and echinostome among 4 species of snail with a prevalence of 29, 23 and 3% respectively. *Melanoides tuberculata* snail was the most susceptible host for cercariae infection. Four species of metacercariae, *Haplorchis taichui*, *Stellantchasmus falcatus*, *Haplorchoides* sp and *Centrocestus caninus*, were found with a prevalence of 67, 25, 60 and 20%, respectively. The Siamese mud carp (*Henicorhynchus siamensis*) was the most susceptible fish host for *H. taichui*, and half-beaked fish (*Dermogenys pusillus*) for *S. falcatus* metacercariae infection, whereas *Haplorchoides* sp and *C. caninus* were concomitantly found in *Puntius brevis*. HAT-RAPD profile confirmed that pleurolophocercus cercariae found in *Melanoides tuberculata* from Mae Taeng District belonged to *H. taichui* and in *Tarebia granifera* from Mueang District were *S. falcatus*.

INTRODUCTION

In Thailand, heterophyid flukes, *Stellantchasmus falcatus*, *Centrocestus caninus* and *Haplorchis taichui*, were reported as endemic species in the northern region (Sripalwit *et al*, 2003). Humans become infected with these parasites by eating undercooked freshwater fish containing metacercariae (Kumchoo *et al*, 2003). Fish-eating animals, including dog, cat and rodent, can also become infected and can serve as reservoirs of infection (Le *et al*, 2006). Because of the sympatric relationship found

among several metacercarial species in the same fish and snail hosts including their morphology, which is particularly similar in the egg forms and larval stages, it is difficult to distinguish such parasites from one another by standard methods. Consequently, specific and accurate detection is needed for better definition and epidemiological control program.

In recent years, molecular approaches using PCR method have been developed for specific detection of different parasites species, *viz.* PCR and filter-hybridization have been introduced to detect bird schistosome cercariae in lakes (Hertel *et al*, 2002), snail host, and in fecal, water and plankton samples (Hamburger *et al*, 1998; Pontes *et al*, 2002). In Thailand, specific DNA probes have been developed for the detection of

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O. viverrini, (Sermsawan *et al*, 1991) and *Paragonimus heterotremus* (Intapun *et al*, 2005). Additionally, a PCR-RFLP methods has been developed to detect cercariae of the large liver fluke, *Fasciola hepatica* infecting *Lymnaea columella* snail (Magalhães *et al*, 2004). Due to their rapidity, accuracy and specificity, PCR-based methods have also been introduced for use as coprodiagnosis in comparison with standard methods in the detection of several parasites, such as *Echinococcus multilocularis* in the definitive host (Dinkel *et al*, 1998) and *O. viverrini* (Wongratanacheewin *et al*, 2002).

This study is aimed to identify trematode species at larval stages in intermediate hosts (cercariae in snail and metacercariae in fish) using a combination of standard and molecular (HAT-RAPD) methods, which should be useful to demonstrate the epidemiological situation and in application in control programs.

MATERIALS AND METHODS

Snails and fish were collected from paddy fields in 3 districts: Mueang, Mae Taeng and Mae Rim of Chiang Mai Province during April-July 2008. For the investigation of larval trematode infection, standard (cracked for snails and enzymatically digested for fish) and molecular methods were performed.

HAT-RAPD PCR

Parasite genomic DNA was extracted and purified from adult worms using Dneasy Tissue Kit (QIAGEN) according to instructions of manufacturer. Extracted genomic DNA was diluted to a working concentration of 50 ng/μl and stored at -20°C until used.

A high annealing temperature-random amplified polymorphic DNA (HAT-RAPD) technique (Anuntalabhochai *et al*, 2000)

was performed using commercially available arbitrary 10-mer primers (Operon technology, USA). HAT-RAPD PCR reaction was carried out in a final volume of 20 μl. The reaction was performed in MyCycler™ Thermocycler (Bio RAD) as follows: 1 cycle of 95°C for 5 minutes, 30 cycles of 95°C for 45 seconds, 48°C for 45 seconds, 72°C for 2 minutes; and 1 cycle of final extension at 72°C for 7 minutes. HAT-RAPD PCR products were separated by 1.4% agarose gel electrophoresis, stained with ethidium bromide and photographed (Kodak digital camera Gel Logic 100).

RESULTS

Three types of cercariae were found, pleurolophocercus, cotylocercous and echinostome, among 3 species of snail (*Melanooides tuberculata*, *Tarebia granifera*, *Bithynia funiculata*) with a prevalence of 29, 23 and 3%, respectively (Table 1) *M. tuberculata* snail was the highest susceptible host for cercariae infection. Interestingly, it was also found that pleurolophocercus showed a high infection capability as it was found in both *Melanooides* and *Tarebia* snails.

Metacercariae of four parasite species, *Haplorchis taichui*, *Stellantchasmus falcatus*, *Haplorchoides* sp and *Centrocestus caninus*, were found among 3 fish species (*Henicorhynchus siamensis*, *Dermogenys pusillus* and *Puntius brevis*) with a prevalence of 67, 25, 60 and 20%, respectively (Table 2). The Siamese mud carp, *Henicorhynchus siamensis*, was the most susceptible fish host for *H. taichui*, and half-beaked fish, *Dermogenys pusillus*, for *S. falcatus* metacercariae infection, whereas *Haplorchoides* sp and *C. caninus* were concomitantly found with high specificity in *Puntius brevis*. *Haplorchoides* sp was also found in *H. siamensis* indicating a higher infection capability than others.

Table 1
Prevalence of cercariae infection among snail intermediate host collected from paddy field in 3 districts of Chiang Mai Province.

Cercariae	No. infected/examined	% Prevalence
Pleurolophocercous	25/90	29
Cotylocercous	21/90	2
Echinostome	3/90	3

Table 2
Prevalence of metacercariae infection among fish intermediate host collected from paddy field in 3 districts of Chiang Mai Province.

Metacercariae	No. infected/examined	% Prevalence
<i>Haplorchis taichui</i>	27/40	67
<i>Stellantchasmus falcatus</i>	10/40	25
<i>Haplorchoides</i> sp	24/40	60
<i>Centrocestus caninus</i>	8/40	20

HAT-RAPD profile confirmed that pleurolophocercous cercariae found in *Melanoides tuberculata* from Mae Taeng District (Fig 1, lane 11) belonged to *H. taichui* (Fig 1, lane 3), whereas pleurolophocercous in *Tarebia granifera* from Mueng District (Fig 1, lane 12) was identified as *S. falcatus* (Fig 1, lane 4). From HAT-RAPD profile, it also was found that pleurolophocercous cercariae could infect different snail hosts and importantly, they could come from more than one species. This study revealed that *S. falcatus* and *H. taichui* were developed from pleurolophocercous cercariae (Fig 1).

DISCUSSION

This study showed that *M. tuberculata* snail was the most susceptible host for cercariae infection and pleurolophocercous showed high infection capability because it was found in both *Melanoides* and *Tarebia*

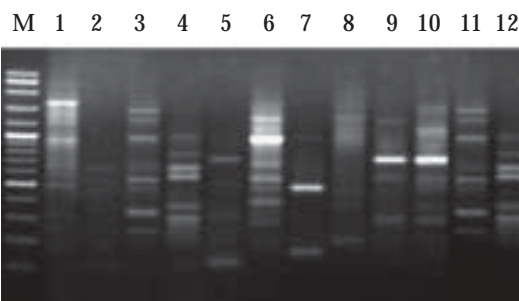


Fig 1-HAT-RAPD profile generated by OPN-09 primer. Lane 1, *Haplorchoides* sp; lane 2, *Ganeo tigrinus*; lane 3, *Haplorchis taichui*; lane 4, *Stellantchasmus falcatus*; lane 5, *Opisthorchis viverrini*; lane 6, *Centrocestus caninus*; lane 7, *Diplodiscus* sp; lane 8, *Orthocoelium streptocoelium*; lane 9, *Paramphistomum epiclitum*; lane 10, *Fischoederius elongatus*; lane 11, pleurolophocercous cercariae (in *Tarebia* snail); lane 12, pleurolophocercous cercariae (in *Melanoides* snail). DNA was extracted from adult worm in lanes 1-10, and from cercariae in lanes 11-12.

snails. For metacercariae infection, Siamese mud carp (*H. siamensis*) was the most susceptible fish host for *H. taichui*, half-beaked fish (*D. pusillus*) for *S. falcatus* metacercariae infection, and *Haplorchoides* sp and *C. caninus* were concomitantly found in *P. brevis*. It can be assumed that pleurolophocercus is serving as a dominant cercarial type found in the study area, while most of the metacercariae belong to family Heterophyidae, similar to results of Sripalwit *et al* (2003); Kumchoo *et al* (2005) and Boonchot *et al* (2005).

HAT-RAPD profile confirmed that pleurolophocercus cercariae found in *Melanooides tuberculata* from Mae Taeng District, Chiang Mai Province belonged to *H. taichui*, whereas pleurolophocercus in *Tarebia granifera* from Mueang District were *S. falcatus*, which provides evidence to support that most trematodes endemic in northern Thailand are in the family Heterophyidae. Chuboon and Wongsawad (2003) have reported that trematode's eggs present in human stool of villagers in Chom Thong District, Chiang Mai Province are *H. taichui*.

It can be concluded that the molecular method yields significant efficiency in the detection and identification of larval trematode infecting intermediate hosts. However, it should be performed together with conventional or standard methods for better definition of the epidemiological situation.

ACKNOWLEDGEMENTS

Authors wish to thank the Commission on Higher Education, for financial support through its PhD research program. Special thanks are extended to the Applied Technology in Biodiversity Research Unit, Institute for Science and Technology Research, Chiang Mai University, the Parasitology Research Laboratory and the Economic Plant Genome Research and Service Center, Department of Biology, Faculty of Science,

Chiang Mai University for providing instrumental facilities.

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