# A SURVEY OF HELMINTHS IN CYPRINOID FISH FROM THE MAE NGAD SOMBOONCHON RESERVOIR, CHIANG MAI PROVINCE, THAILAND

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Abstact. Five species of cyprinoid fish, *Puntioplites proctozysron, Hampala macrolepidota, Labiobarbus siamensis, Barbodes gonionotus,* and *Henicorhynchus siamensis,* were collected from Mae Ngad Somboonchon Reservoir, Chiang Mai Province from October 2002 to September 2003 and examined for parasites. Fourteen species of helminths were discovered: ten species of monogenea, *Dactylogyrus macrolepidoti, D. protozysron, D. puntioplites, D. quadribrachiatus, Dactylogyrus* sp I, II, III, *Gyrodactylus* sp, *Paradiplozoon kamang,* and *P. krasoopensis*; three species of metacercariae, *Centrocestus caninus, Haplorchis taichui,* and *Haplorchoides* sp and one species of nematode: *Rhabdochona* sp. The overall prevalence was 79.36% (373/470). The highest prevalence was found in *Puntioplites proctozysron* (90%, 81/90), and the lowest prevalence in *Labiobarbus siamensis* (60.64%, 57/94). Monogenea and metacercariae were found with the highest and lowest infection rates in the cool-dry and rainy seasons, respectively. Metacercariae of *Centrocestus caninus* and *Haplorchis taichui* are infectious to humans.

#### INTRODUCTION

Mae Ngad Somboonchon Reservoir is located in Mae Tang district, Chiang Mai Province, northern Thailand. It serves as an important area for both aquatic culture and fisheries in northern Thailand. There are many aquatic animals, especially cyprinoid fish, which serve as the intermediate hosts of many parasites that are dangerous to animals and humans.

In Thailand, helminth parasites such as *Diplozoon* sp, *Acanthosentis* sp, and *Senga* sp have been found in *Hampala macrolepidota* collected from Vajiralongkorn Reservoir (Soonthornsatit, 1982). *Dactylogyrus puntioplites* was the most common species found in *Puntioplites proctozysron* (34.14%) from Srinakarin Reservior, Kanchanaburi Province (Theanhom, 1997). A new species, *Thaparogyrus jullieni*, was found in *Cirrhinus jullieni* from the Suphan Buri River, Suphan Buri Province (Purivirojkul, 1999). Infective stages (metacercarriae) of *Haplorchis* spp and *Opisthorchis viverrini* were found in cyprinoid fish in Khon Kaen Province, northeast Thailand (Srisawangwong *et al*, 1997) and in cultured

Correspondence: K Boonchot, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand. Tel: 66 (0) 5389-2259 cyprinoid fish in Chiang Mai Province, northern Thailand (Khamboonruang *et al*, 1997). Recently, *Centrocestus caninus, Haplochis taichui*, and *Stellantchasmas falcatus* were recovered from cyprinoid fish collected from Mae Sa Stream, Mae Rim district, Chiang Mai Province (Wongsawad *et al*, 2000). These three species have been reported to infect humans (Tantachamrun and Kliks, 1974; Radomyos *et al*, 1994, 1998). Nematodes in freshwater fish have been found in Mae Sa Stream, Chiang Mai Province by Kumchoo (2000). The dominant species of nematode in Chiang Mai Province was *Rhabdochona* spp (Moravec and Nagasawa, 1998; Sanchez-Alverez *et al*, 1998).

Our study was to determine the prevalence of helminth infection in five species of cyprinoid fish from Mae Ngad Somboonchon Reservoir, Chiang Mai Province.

## MATERIALS AND METHODS

Thirty individuals of each cyprinoid fish were collected during each season. These cyprinoids were: Puntioplites proctozysron, Hampala macrolepidota, Labiobarbus siamensis, Barbodes gonionotus, and Henicorhynchus siamensis; collected at Mae Ngad Somboonchon Reservoir, Chiang Mai Province from October 2002 to September 2003. These fish were collected by fisherman using nets, placed in an ice box and transported to our laboratory. All the fish were identified by illustrations distrib- 8 70% uted by the Department of Fisheries, Ministry of Agriculture and Co-operatives, Thailand. The 250% scales, fins, muscles, and body cavity, including visceral organs of each specimen, were 🛎 30% examined for helminths under a stereomicroscope. Muscles were digested using an acid pepsin solution (1 ml conc. hydrochloric acid, 1 g pepsin, 99 ml 0.85% sodium chloride solution for 1  $^{1}/_{2}$  hours at 37°C. The digested material was then rinsed with 0.85% sodium chloride solution and examined for metacercariae. The helminths were removed, counted, fixed in 4% formalin, and stained with Borax's carmine or hematoxylin, dehydrated in an alcohol series, and mounted with Permount. The species were identified after Yamaguti (1958, 1963), Pearson (1964), Kliks and Tantachamrun (1974), Pearson and Ow-Yang (1982), Radomyos et al (1990), and Kaewviyudth (1998).

#### RESULTS

Fourteen species of helminths were recorded. This included ten species of monogenea: *Dactylogyrus macrolepidoti, D. protozysron, D. puntioplites, D. quadribrachiatus, Dactylogyrus* sp I, II, III, *Gyrodactylus* sp, *Paradiplozoon kamang,* and *P. krasoopensis*. Three species of metacercariae were also found: *Centrocestus caninus, Haplorchis taichui,* and *Haplorchoides* sp; and one species of nematode, *Rhabdochona* sp a nematode.

The overall prevalence of infection was 79.36% (373/470). The most helminths were recovered from Puntioplites proctozyron (90%, 81/90), and the least from Labiobarbus siamensis (60.64%, 57/94). Puntioplites proctozysron was infected with Dactylogyrus protozysron, D. puntioplites, Haplorchis taichui, and Paradiplozoon kamang, with an infection rate of 90%. Hampala macrolepidota was infected with Dactylogyrus macrolepidoti, D. quadribrachiatus, Haplorchis taichui, Paradiplozoon krasoopensis, and Haplorchoides sp, with an infection rate of 87.88%. Labiobarbus siamensis was infected with Dactylogyrus spl, Centrocestus caninus, and Haplorchis taichui,

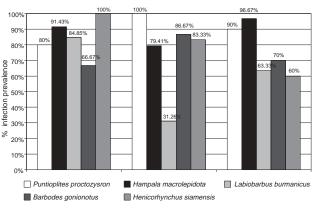


Fig 1–Prevalence of helminthic infections in five species of cyprinoid fish in the cool-dry, hot and rainy seasons.

with an infection rate of 60.64%. Barbodes gonionotus was infected with Dactylogyrus sp II, Gyrodactylus sp, Haplorchis taichui, and Rhabdochona sp with an infection rate of 74.44%. Henicorhynchus siamensis was infected with Dactylogyrus sp III, Haplorchis taichui, and Rhabdochona sp with an infection rate of 82.47%. Monogenea included Dactylogyrus sp, Gyrodactylus sp and Paradiplozoon sp which were recovered from the gills of cyprinoid fish. Metacecariae of Centrocestus caninus, Haplorchis taichui, and Haplorchoides sp were present on the scales and in the muscles. Rhabdochona sp were found in the intestines (Table 1).

In the cool-dry season, the overall infection prevalence was (85.45%, 141/165). The highest prevalence was found in *Henicorhynchus siamensis* (100%, 37/37) and the lowest in *Barbodes gonionotus* (66.67%, 20/30). In the hot season, the overall prevalence was (76.13%, 118/155), in which *Puntioplites proctozysron* had the highest infection rate (100%, 30/30) and *Labiobarbus siamensis* had the lowest (31.26%, 10/31). In the rainy season, the overall prevalence was (76.0%, 114/150), with the highest prevalence in *Hampala macrolepidota* (96.67%, 29/30) and the lowest in *Henicorhynchus siamensis* (60%, 18/30) (Fig 1).

### DISCUSSION

Three species of monogenea and one species of a metacercarial stage trematode were found in *Puntioplites proctozysron*. Previously, six species of monogeanea (Dactyrogyrus hamatus, D. kamang, D. protozysron, D. puntioplites, D. thailandensis, and Paradiplozoon kamand) were found in this fish (Chinabut and Lim, 1994; Kaewviyudth, 1998; Theanhom, 1997). Kosalanant (1987) and Sukontason et al (1999) found metacercariae of *Centrocestus* caninus, Haplorchis taichui, and Opisthorchis viverrini in that fish. Kosalanant (1987) recorded Spinitectus sp (nematode) and Acanthosentis sp (Acanthocephala), in the intestines and spleen, which were not found in our study.

In Hampala macrolepidota, we found three species of monogenea and one species of metacercaria, while other researchers (Kaewviyudth, 1998; Kosalanant, 1987; Lim, 1987) found five species of monogenea (Dactyrogyrus anchorobustus, D. hampali, D. macrolepidoti, D. quadribrachiatus, and Paradiplozoon soinogkaonensis). The metacercaria of H. taichui have been recovered, as seen in some districts of Chiang Mai and Lumphun Provinces (Namue et al, 1998; Sukontason et al, 1999). We did not find nematodes or acanthocephala, but these had been found by Kosalanant (1987). Kosalanant (1987) also found Spinitectus sp, three unidentified species of nematodes, and Acanthosentis sp from Ubonrut Reservoir, Khon Kaen Province.

In Labiobarbus siamensis, we found Dactylogyrus sp and metacercariae of C. caninus and H. taichui. Sukontason (1999) reported H. pumilio and O. viverrini in this fish in Ban Pao district, Chiang Mai Province.

We recovered two species of monogenea, one species of metacercariae, and one species of nematode in Barbodes gonionotus. Previous studies recorded eight species of monogeanea in this fish (Dactyrogyrus lampan, D. kanchanaburiensis, D. pseudosphyrna, D. tapienensis, D. tonguthaii, D. siamensis, and D. viticulus) (Chinabut and Lim, 1993; Kaewviyudth, 1998). Srisawangwong (1997) found metacercariae of Centrocestus formosanus. Haplorchis pumilio, and H. taichui in the same fish from Khon Kaen Province, which is similar to our findinas.

In Henicorhynchus siamensis, we found one species of monogeanea, one metacercariae, and

Chiang Mai Province.						
Host	Number of infected fish/examined	Prevalence (%)	Helminth	Site of infection		
Puntioplites proctozysr	on 81/90	90.00	Dactylogyrus protozysron D. puntioplites Haplorchis taichui Paradiplozoon kamang	Gills Gills Scales and muscle Gills		
Hampala macrolepidot	a 87/99	87.88	Dactylogyrus macrolepidoti D. quadribrachiatus	Gills Gills		

Table 1					
Prevalence of helminths in five species of cyprinoid fish from Mae Ngad Somboonchon Reservior,					
Chiang Mai Province.					

	fish/examined	(%)		
Puntioplites proctozysron	81/90	90.00	Dactylogyrus protozysron	Gills
			D. puntioplites	Gills
			Haplorchis taichui	Scales and muscle
			Paradiplozoon kamang	Gills
Hampala macrolepidota	87/99	87.88	Dactylogyrus macrolepidoti	Gills
			D. quadribrachiatus	Gills
			Haplorchoides sp	Scales and muscle
			Haplorchis taichui	Scales and muscle
			Paradiplozoon krasoopensis	Gills
Labiobarbus burmanicus	57/94	60.64	<i>Dactylogyrus</i> sp I	Scales and muscle
			Centrocestus caninus	Gills
			Haplorchis taichui	Scales and muscle
Barbodes gonionotus	67/90	74.44	Dactylogyrus sp II	Gills
			<i>Gyrodactylus</i> sp	Gills
			Haplorchis taichui	Scales and muscle
			<i>Rhabdochona</i> sp	intestine
Henicorhynchus siamensis	is 80/97	82.47	<i>Dactylogyrus</i> sp III	Gills
			Haplorchis taichui	Scales and muscle
			<i>Rhabdochona</i> sp	Intestine
Total	373/470	79.36		

one nematode. Kosalanant (1987) found two species of *Dactylogyrus* and metacercariae of *O. viverrini* in this fish from Khon Kae in six districts in Chiang Mai Province, which agrees with our results.

Dactylogyrus infestation causes gill hyperplasia, proliferation of mucous cells, epithelial edema, hyperemia of gills, cytolysis, and finally cell necrosis. Migration of inflammatory cells to Dactylogyrus infected areas was noticed by Kaewviyudth (1998).

We found three species of metacercariae: C. caninus, H. taichui, and Haplorchoides sp. Most metacercariae were found on scales and in muscles, as reported by Namue et al (1998). Kabkaew (2001) and Tesana et al (1985) found that the caudal fin had the highest number of metacercariae and the anal fin had the fewest. Metacercariae of *H. taichui* were observed by Ooi et al (1997) to be mostly in the tail region of fish. H. taichui was still the most abundant metacercariae found in our study. C. caninus and H. taichui are heterophyid flukes reported to infect humans (Martin, 1958; Pearson, 1964; Radomyos et al, 1998; Tantachamrun and Kliks, 1978). Heterophyid flukes produce mild mucosal irritation accompanied by intestinal colicky pains and mucous diarrhea (Beaver et al, 1984)

*Rhabdochona* sp were collected from the intestines of *Barbodes gonionotus* and *Heni-corhynchus siamensis*, as reported by Kosalanant (1987), who also found *Rhabdochona chodukini*, *R. denudata* and *Rhabdochona* sp in *Cyclo-cheilichthys apogon* and *Osteochilus hasselti*. Moravec and Nagasawa (1998) studied helminths from the rare endermic catfish, *Liobagrus reini*, in Japan, while Sanchez-Alverez *et al* (1998) found *R. lichtenfelsi* in *Allophorus robustus* and *Goodea atripinnis* in two Mexican lakes. Kumchoo (2000) recorded four species of *Rhabdochona* in freshwater fish from Mae Sa Stream, Chiang Mai Province.

Kaewviyudth (1998) indicated that the seasonal dynamics of most monogenean populations do not differ significantly. We found the highest infection rates for monogenea and metacercariae in the cool-dry season, and the lowest in the rainy season, which agrees with Srisawangwong (1997) and Sukontason (2001). The high infection rate in *H. taichui* in the cooldry season may be due to the previous rainy season. In the rainy season, water may flood fecal material, including parasite eggs, into water resources. This parasite requires more than 6 weeks in intermediate host snails to reach the cercarial stage, and 2-3 weeks in fish to reach the metacercarial stage (Faust and Nishigori, 1926). The 2 month developmental time correlates with the high incidence in the following cool-dry season (Sukontason *et al*, 1999).

Our study showed a high prevalence of helminths in cyprinoid fish in Chiang Mai Province, especially monogeanea and metacercariae, with the highest infection rates in the cool-dry season. This may be caused by unsanitary rural latrines. Eggs and larvae of helminths can contaminate reservoirs by running water and precipitation. Parasites can infect humans who eat undercooked fresh-water fish. The data from our study can help rural people who use this contaminated water prevent parasitic infections in future.

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## REFERENCES

- Beaver PC, Jung RC, Cupp EW. Clinical Parasitology. 9<sup>th</sup> ed. Philadelphia: Lea and Febiger 1984.
- Chinabut S, Lim LS. Five new species of Dactylogyrus Diesing, 1850 (Monogenea) from *Puntiolites protozysron* (Bleeker) (Cyprinidae) of Thailand. *Raff Bull Zool* 1994; 42: 885-92.
- Faust EC, Nishigori M. The life cycles of two new species of heterophyidae, parasitic in mammals and birds. *J Parasitol* 1926; 13: 91-132.
- Kaewviyudth S. Species and Distribution of Monogenean Parasite of Some Cyprinid Fish. Graduate School, Kasetsart University, 1998: 307 p. PhD Thesis.

- Khamboonruang C, Kaewvichit R, Wongworapot K, *et al.* Application of hazard analysis critrical contro point (HACCP) as a possible control measure for *Opisthorchis viverrini* infection in cultured carp (*Puntius gonionotus*). Southeast Asian J Trop Med Public Health 1997; 28: 65-72.
- Kliks M, Tantachumrun T. Heterophyid (Trematode) parasite of cats in north Thailand, with notes on a human case found at necropsy. *Southeast Asian J Trop Med Pubic Health* 1974; 5: 547-55.
- Kosalanant V. Studies on Helminth Parasite of some Fish Species Belonging to the Familly Cyprinidae in Ubonrut Reservoir, Changwat Khon Kaen. Chiang Mai, Kasetsart University, 1987: 180 p. MSc Thesis.
- Kumchoo K. Survey of Nematode and SEM of Tegument in Freshwater Fish from Mea Sa Stream, Doi Suthep-Pui National Park, Chiang Mai Province. Chiang Mai, Chiang Mai University, 2000. 168 p. MSc Thesis.
- Lim LHS. Distribution and Diversity of Monogeneans in Freshwater Fishes of Peninsular Malaysia. Kuala Lumpur, University of Malaysia, 1987. 186 p. PhD Thesis.
- Martin WE. The life histories of some Hawaiian heterophyid trematodes. *J parasitol* 1958; 44: 305-23.
- Moravec F, Nagasawa K. Heminth parasites of the rare endemic catfish, *Liobagrus reini* in Japan. *Folia Parasitol* 1998; 45: 283-94.
- Namue C, Rojanapaibul A, Wongsawad C. Occurrnce of two heterophyid metacercaria Haplorchis and Haplorchoides in cyprinoid fish of some districts in Chiang Mai and Lumphun province. *Southeast Asian J Trop Med Public Health* 1998; 29: 401-5.
- Ooi HK, Chen CL, Lin SC, Tung KC, Wang IS, Kamiya. Metacercariae in fish of Sun Moon Lake whish is an endemic area for Clonorchis sinensis in Taiwan. *Southeast Asian J Trop Med Public Health* 1997; 28(suppl 1): 222-3.
- Pearson JC, Ow-Yang CK. New species of *Haplorchis* from Southeast Asia, together with keys to the *Haplorchis* group of heterophyid trematodes of the region. *Southeast Asian J Trop Med Public Health* 1982; 13: 35-60.
- Pearson JC. A revision of the subfamily Haplorchinae Looss, 1899 (Trematoda: Heterophyidae). *Parasitology* 1964; 54: 601-76.
- Purivirojkul W. Parasite of Pla Soi Khao *Cirrhinus jullieni* Sauvage from Suphan Buri River, Changwat Suphan Buri. Bangkok, Kasetsart University, 1999: 95 p. MSc Thesis.
- Radomyos P, Charoenlarp P, Radomyos B, Tungtrongchir A. Two human cases of *Stellantchasmus falcatus* (Trematode: Heterophyidae) infection in Northeast-

ern Thailand. Jpn J Parasitol 1990; 39: 7-11.

- Radomyos B, Wongsaroj T, Wilairatana P, *et al.* Opisthorchiasis and intestinal fluke infections in northern Thailand. *Southeast Asian J Trop Med Pubic Health* 1998; 29:123-7.
- Radomyos P, Radomyos B, Tungtrongchir A. Multiinfection with helminths in adults from northeast Thailand as determined by post-treatment fecal examination of adult worm. *Trop Med Parasitol* 1994; 45: 133-5.
- Sanchez-Alverez A, Garcia-Prieto L, Perez-Ponce de Leon G. A new species of *Rhabdochona Railliet*, 1916 (Nematode: Rhabdochonidae) from endermic Goodeids (Cyprinodontifomes) from two Mexican lakes. *J Parasitol* 1998; 84: 840-45.
- Soonthornsatit, Parasites of the Pla Kasoop Keid (*Hampala macrolepidota* van Hasselt) in Vajilalongkorn Reservoir. Graduate School, Kasetsart University, 1982: 97 p. MSc Thesis.
- Srisawangwong T, Sithihaworn P, Tesana S. Metacercariae isolated from cyprinoid fish in Khon Kaen district by digestion technique. *Southeast Asian J Trop Med Public Health* 1997; 28 (suppl 1): 224-6.
- Sukontason K, Piangjai S, Muangyimpong Y, Sukontason K, Methanitikorn R, Chaithong U. Prevalence of trematode metacercariae in cyprinoid fish of Ban Pao district, Chiang Mai Province, northern Thailand. Southeast Asian J Trop Med Public Health 1999; 30: 365-70.
- Sukontason KL, Sukontason K, Boonsriwong N, Chaithong U and P. Intensity of trematode metacercariae in cyprinoid fish in Chang Mai Province, northern Thailand. *Southeast Asian J Trop Med Public Health* 2001; 32 (suppl 1): 214-17.
- Tantachamrun T, Kliks M. Heterophyid infection in human ileum: report of three cases. *Southeast Asian J Trop Med Public Health* 1978; 9: 228-31.
- Tesana S, Kaewkes S, Srisawangwong T, Phinlaor S. Distribution and density of *Opisthorchis viverrini* metacercariae in cyprinoid fish from Khon Kaen Province. *J Parasit Trop Med Assoc Thai* 1985; 8:36-9.
- Theanhom S. Parasite of Pla-Kamung *Puntioplitrs protozysron* (Bleeker) in Srinakarin Reservior Changwat Kanchanaburi. Bangkok, Kasetsart University, 1997: 144 p. MSc Thesis.
- Wongsawad C, Rojanapaibul A, Mhad-arehin N, *et al.* Metacercariae from freshwater fishes of Mae Sa Stream, Chiang Mai. Thailand. *Southeast Asian J Trop Med Public Health* 2000; 31 (suppl 1): 54-7.
- Yamaguti S. Systema Helminthum. Vol I. The digestic trematode of vertebrate, part I and II. New York Interscience Publishers, 1958: 979 p.
- Yamaguti S. Systema Helminthum. Vol IV. Monogenea and Aspidocotylea. Interscience Publishers, 1963: 699 p.