Microsc. Microanal. Res. 2019, 32(1) 7-11

Microscopy and Microanalysis Research
- The Journal of The Microscopy Society of Thailand

Journal Home Page: https://www.tci-thaijo.org/index.php/mmres/

Microsc. Microanal. Res.

Prevalence and Morphological Changes on Fish Gills of Cyprinoid Fish Infected with *Centrocestus formosanus* Metacercariae from Li River, Lamphun Province

Ratchadaporn Kuntatum[1], Chalobol Wongsawad[1, 2*], Preeyaporn Butboonchoo[1, 2] and Pheravut Wongsawad[1, 3]

[1] *Department of Biology, Faculty of Science, Chiang Mai University, Muang, 50200, Thailand*
[2] *Environmental Science Research Center (ESRC), Chiang Mai University, Muang, 50200, Thailand*
[3] *Center of excellence in Bioresources for Agriculture, Industry and Medicine, Faculty of Science, Chiang Mai University, Muang, 50200, Thailand*

*Corresponding author's e-mail address: wchalobol@gmail.com (C.Wongsawad)

ARTICLE INFO

Article history
Submitted: 10 April 2019
Revised: 14 July 2019
Accepted: 19 July 2019
Available online: 26 September 2019

Keywords:
Prevalence; *Centrocestus formosanus*; Gill; Metacercaria; *Parambassis siamensis*; *Raiamas guttatus*; Lamphun

ABSTRACT

Centrocestus formosanus has been reported to infect the natural fish gill in Northern Thailand. Their infection can cause disease and death. We aimed to study the prevalence and observe morphological changes on fish gill of Siamese glassfish (*Parambassis siamensis*) and Burmese Trout (*Raiamas guttatus*) infected with *C. formosanus* metacercariae by using light microscope and long-section. Fish specimens were caught from Li River in Lamphun province, the North of Thailand. The result revealed the oval-shaped metacercaria attaching on both fish species gills. The metacercaria was identified to be *C. formosanus* based on 32 circumoral spines and X-shaped excretory bladder. The prevalence, intensity and abundance of *C. formosanus* infection in *Parambassis siamensis* were 74.3%, 25.6 metacercaria/fish and 19 metacercaria/fish, respectively. The *C. formosanus* infection of *Raiamas guttatus* was lower than *Parambassis siamensis* in all parameters that included 54.3% of prevalence, 5.7 of intensity and 3.1 of abundance. The infection of *C. formosanus* in *R. guttatus* was the first record in the northern part of Thailand. For the pathological observation on fish gill tissue in both species, the result showed the *C. formosanus* metacercaria attached on the middle of primary gill lamella. The metacercaria was surrounded by thick refractile capsule which caused the fusion of two primary gill lamellae. This gill lamella was thick and distorted in shape that affected the cartilage proliferation, hyperplasia and reduction of gas on the surface. Finally, these morphological changes leaded to death in fish.

INTRODUCTION

Centrocestus formosanus (Digenea: Heterophyidae) is a small intestinal trematode of fish-eating birds (ducklings, and chickens) and mammals (rats, rabbits, cats, dogs, and foxes) [1]. They have been reported to infect humans in Taiwan, Japan, and Lao PDR [2, 3]. The metacercaria of *C. formosanus* has been recorded in various species of freshwater fish act as the second intermediate host [2, 4, 5]. In the northern part of Thailand, there are several freshwater fish particularly cyprinoid fish infected with *C. formosanus* metacercaria included; *Esomus metallicus*, *Puntius brevis*, *Parambassis siamensis*, *Carassius auratus*, *Cyprinus carpio*, *Danio rerio*, *Puntigrus tetrazona* [6, 7]. The presence of *C. formosanus* metacercaria on the fish gill is one of the reasons for the reduction of fish production in aquaculture [8-10]. Heavy infections result in serious tissue replacement, secondary bacterial infection and death [8, 11] The histology of *C. formosanus* infection in fish gill has been studied in many previous reports [8-12]. However, the histological study of infected fish gill has not been reported in the northern part of Thailand.

The present study aimed to investigate the prevalence, intensity and abundance of *C. formosanus* infection in 2 cyprinoid fishes (*Parambassis siamensis* and *Raiamas guttatus*) collected from Li River in Lamphun province. Both species are generally found in Li River and trend to consume in the people lived near this river. Both *P. siamensis* and *R. guttatus* are also an ornamental fish which are cultured for sell in the ornamental fish shop. Additionally, the histological characteristics of infected fish gills were observed using light microscope and longitudinal section. This study is useful for assessment of the epidemiological

situation and prevention of *C. formosanus* infection in both freshwater fish and human.

METHODOLOGY

Seventy cyprinoid fishes were collected form Li River that flow through Koh Thung Man village, Ban Hong district, Lamphun province

(N 18.275072, E 98.822978). The collected cyprinoid fishes were classified into two species; *Parambassis siamensis* (35 specimens) and *Raiamas guttatus* (35 specimens). Each fish gill was gently removed with forceps to examine the metacercaria of *C. formosanus* under light microscope. The number of recovered metacercaria was recorded to calculate the prevalence intensity and abundance according to Margolis et al. (1982).

$$\% \text{ Prevalence} = \frac{\text{Number of infected fishes} \times 100}{\text{Number of examined fishes}}$$

$$\text{Intensity} = \frac{\text{Number of metacercaria}}{\text{Number of infected fishes}}$$

$$\text{Abundance} = \frac{\text{Number of metacercaria}}{\text{Number of examined fishes}}$$

The encysted metacercaria was excysted to observe the morphological characteristic of larval stage. They were prepared for permanent slides. In brief, they were washed in tap water, then fixed and flattened in 4% formalin, stained with Delafield' hematoxylin, dehydrated in alcohol series, cleared in xylene and permanently mounted in permount. Specimens on permanent slides were illustrated using a compound microscope with a drawing tube.

Both fish gills of *P. siamensis* and *R. guttatus* infected with *C. formosanus* metacercaria were observed for morphological changes. The specimens were fixed in 10% formalin, stained with Delafield' hematoxylin, dehydrated in alcohol series, cleared in xylene and permanently mounted in permount for permanent slide preparation. For histological study, some specimens after clearing were subjected to paraffin section for 8 microns, deparaffined in xylene and mounted with permount.

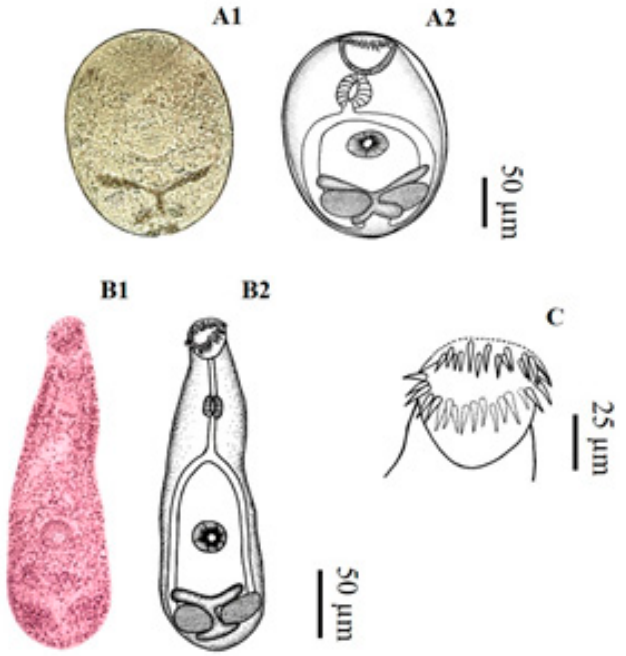

Figure 1. Morphology of recovered C. *formosanus* metacercaria; A. Encysted metacercaria showed the oval shaped with a double layered cystic wall, B. Excysted metacercaria revealed pyriform shape with circumoral spines and X-shaped excretory bladder, C. Oral sucker surrounded by 32 circumoral spines.

RESULTS

Seventy cyprinoid fishes collected from Li River were classified into two species including; *Parambassis siamensis* (35 specimens) and *Raiamas guttatus* (35 specimens). The total 35 *P. siamensis*, 26 specimens were infected with *C. formosanus* metacercaria with the prevalence of 74.3%, intensity of 25.6 metacercaria/fish and abundance of 19 metacercaria/fish (Table 1). *R. guttatus* were infected with the prevalence of 54.3%, intensity of 5.7 metacercaria/fish and abundance of 3.1 metacercaria/fish (Table 1).

The metacercaria of *C. formosanus* was encysted on both gills of *P. siamensis* and *R. guttatus*. The encysted metacercaria (Figure 1A) was oval shape with a double layer cystic wall, possessed a group of circumoral spines and X-shaped excretory bladder. The excysted metacercaria (Figure 1B) was pyriform in shape. Oral sucker terminal was surrounded with 32 circumoral spines (Figure 1C). Prepharynx was present. Pharynx was well developed. Esophagus was short. Caeca enlarged and bifurcated at about midway between oral and ventral sucker. Ventral sucker was smaller than oral sucker, located in the one third of the posterior body. Testes were oval and located opposite in the posterior body. X-shaped excretory bladder was located between testis.

Table 1. The prevalence, intensity and abundance of *C. formosanus* metacercarial infection in *P. siamensis* and *R. guttatus*. N= Number of examined fish

Fish species	P. *siamensis* (N=35)	R. *guttatus* (N=35)
Number of infected fishes	26	19
Number of metacercaria	655	109
%Prevalence	74.3	54.3
Intensity	25.6	5.7
Abundance	19	3.1

Morphological changes of infected gills were observed using light microscope and longitudinal section. The gill structures consisted of primary and secondary gill lamella (Figure 3A and C). The infected gills were found the encysted metacercaria surrounded by thick capsule on the primary gill lamella (Figure 2A and C). The metacercarial embedding region found the fusion and distorting of primary gill lamella (Figure 1B). This region found the cartilage proliferation of primary gill lamella around the metacercaria and the fusion of secondary gill lamella (Figure 2D and 3B, D)

DISCUSSION

The infection of *C. formosanus* metacercaria showed the high prevalence in both fishes. *Parambassis siamensis* was higher (74.3%) than *Raiamas guttatus* (54.3%). The intensity and abundance of *P. siamensis* (25.6, 19 metacercaria/fish, respectively) were also higher than *R. guttatus* (5.7, 3.1 metacercaria/fish, respectively). The result was agreed with the report of Wongsawad et al. (2017) [6] who found

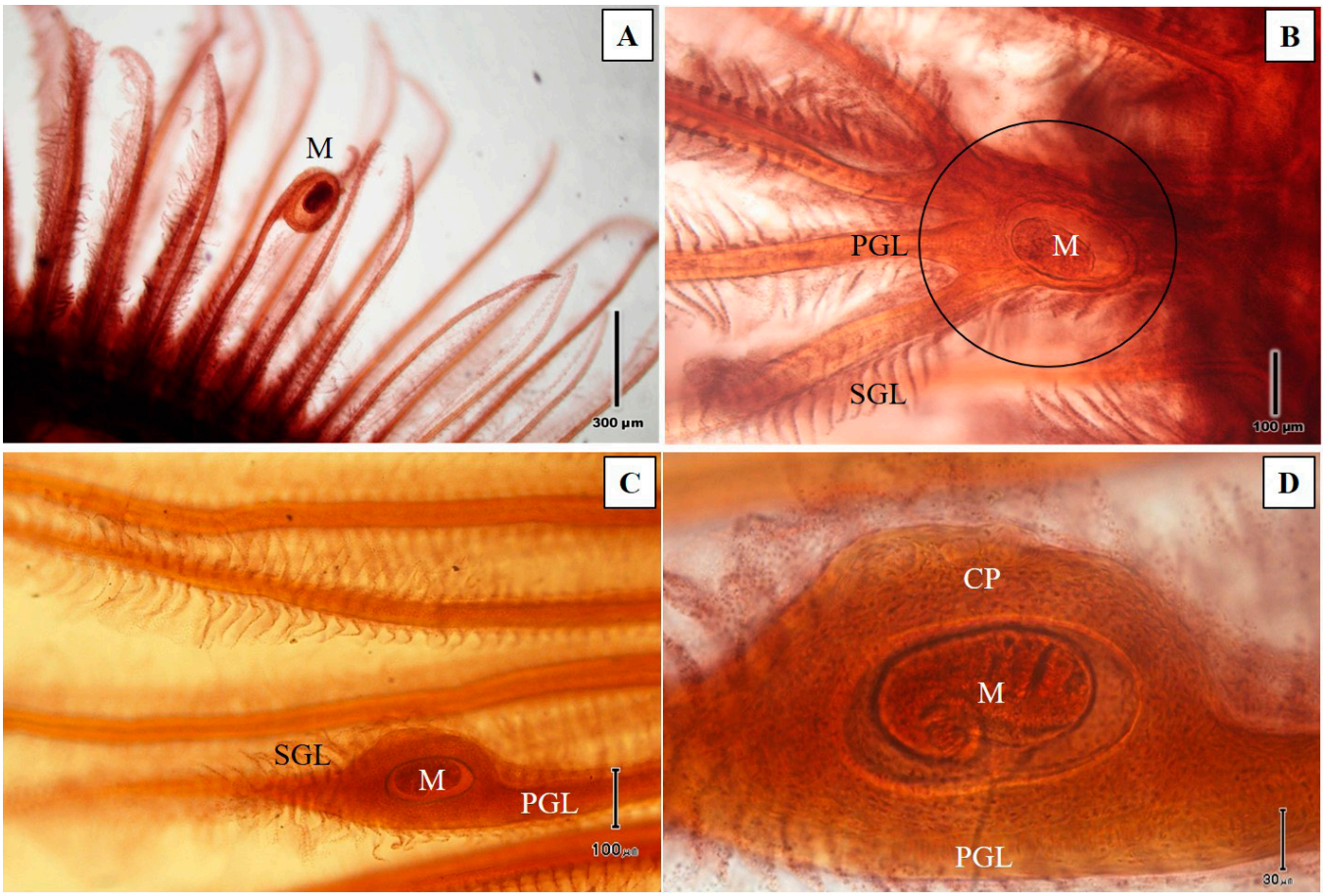

Figure 2. Morphological changes on gills of *P. siamensis* (A and B) and *R. guttatus* (C and D) infected with the metacercaria of *C. formosanus*; A. Metacercaria surrounded by thick capsule on the primary gill lamella, B. Fusion of primary gill lamella (in circle) caused by metacercarial embedding, C. Thickening and distorting of primary gill lamella caused by metacercarial embedding, D. Cartilage proliferation of primary gill lamella surrounding metacercaria.

M= metacercaria, PGL= primary gill lamella, SGL= secondary gill lamella, CP= cartilage proliferation

the *C. formosanus* encysted on the gill of *P. siamensis* collected from Chiang Mai province. Two parameters (prevalence and intensity) of *P. siamensis* were high, which indicated the susceptibility and violence for *C. formosanus* infection. The abundance was also high suggesting that *P. siamensis* habit in Li River flow pass Koh Thung Man village in Ban Hong district has an opportunity to find the large number of metacercaria in the gill. *R. guttatus* was highly infected with *C. formosanus* which was the first record as the second intermediate host in the northern part of Thailand. This finding indicated the increasing of the risk of *C. formosanus* infection to bird and mammal (including human).

The metacercaria encysted on both gills of *P. siamensis* and *R. guttatus* was identified as *C. formosanus* with the main characteristics of 32 circumoral spines and X-shaped excretory bladder. The number of circumoral spines are accepted as the character for distinguishing the species belonging to the genus *Centrocestus* [1]. *C. formosanus* found in this study differed from those with 38-48 spines (*C. nycticoracis*, *C. kurokawai*, and *C. armatus*) and 50-60 spines (*C. polyspinosus*) [13].

The fish host was infected with *C. formosanus* by the attachment and penetration of the cercarial stage. Their cercarial stages habit in the freshwater snails served as the first intermediate host which commonly found in the northern Thailand [14]. The cercaria produce a mucus-like secretion upon attachment, shed their tail, and employ a short period of leech-like creeping behavior, then penetrate and encyst on the surface of primary gill lamella [15]. Morphological changes were observed in the location of metacercaria encysted on the fish gills. Both species, the changes were characterized by hyperplasia of the cartilage of the primary gill lamellae that envelops the metacercaria. The metacercaria was surrounded by thick capsule because of encapsulation of fish host response. The host cyst wall consisted of chondrocytes which were generally elongate adjacent to the parasites [9]. The infected part of gill lamella caused the fusion of two primary gill lamellae. Hyperplasia of the epithelial cell in primary gill lamella results in some part of the secondary gill lamellae. This histological change causes the reduction in gill surface that lead to their decreased function and damages the respiratory system [16]. The same histological changes of *C. formosanus* infection in freshwater fish have been previously described by Blazer and Gratzek (1985) [17], Vélez-Hernández et al. (1998) [18], Yildiz (2005) [19] and Gjurcevic (2007) [9] that reported the *C. formosanus* infection in freshwater fish.

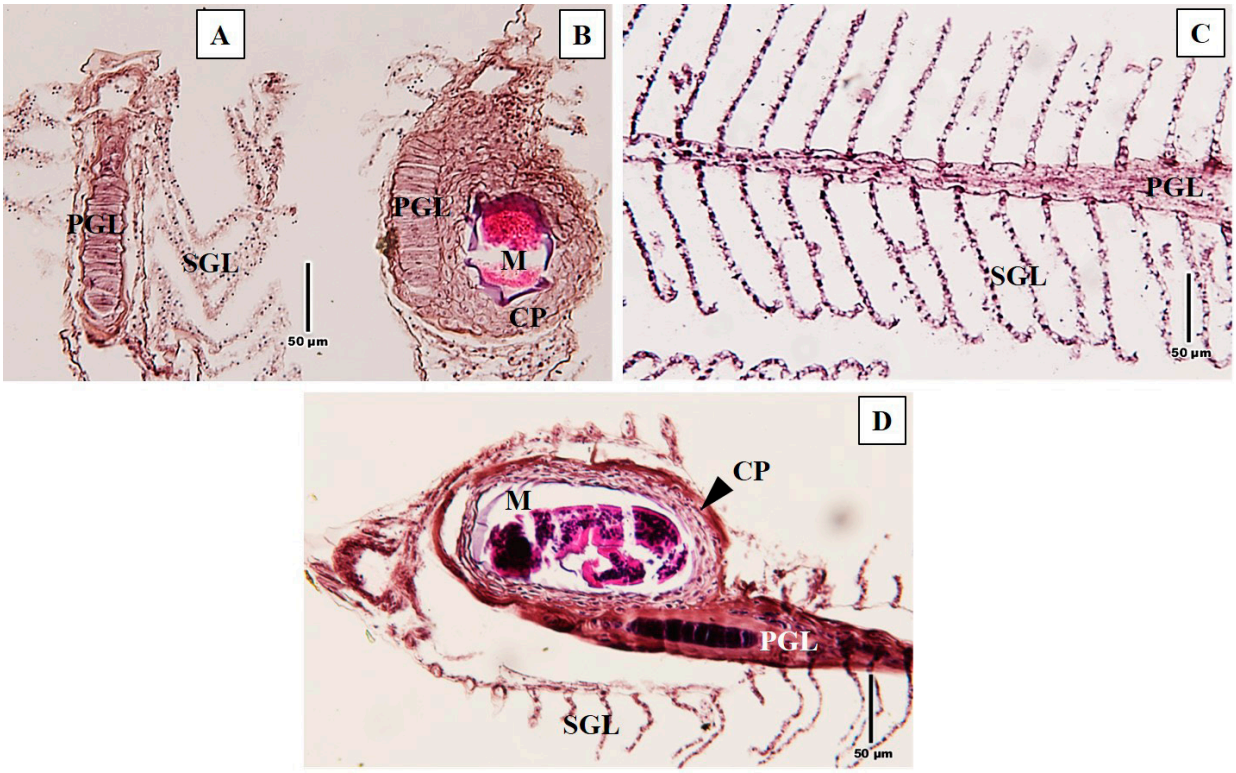

Figure 3. Longitudinal section of gill of *P. siamensis* (A and B) and *R. guttatus* (C and D); A, C. Uninfected gills revealed the gill structure consisting of primary and secondary gill lamella, B, D. Infected gill revealed the cartilage proliferation surrounding metacercaria and distorting of primary gill lamella.

M= metacercaria, PGL= primary gill lamella, SGL= secondary gill lamella, CP= cartilage proliferation

CONCLUSION

The present study has been confirmed that *Centrocestus formosanus* is prevalent in the cyprinoid fish collected from Li River in Lamphun province. *Raiamas guttatus* was firstly reported as the second intermediate of *C. formosanus* in the northern Thailand. So, this finding indicated an increasing of the risk of *C. formosanus* infection to human. Additionally, the histological characters of *C. formosanus* infection in both fish were firstly studied in the northern Thailand which suggested a serious alteration in the fish gill. Freshwater fish in both natural and cultural source should be prevented from this parasite.

ACKNOWLEDGEMENT

Special thanks are extended to Parasitology Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University and Environmental Science Research Center (ESRC), Chiang Mai University for their great assistances.

REFERENCES

[1] H.T. Chen, The metacercaria and adult of *Centrocestus formosanus* (Nishigori, 1924) with notes on the natural infection of rats and cats with *C. armatus* (Tanabe, 1922), *J Parasitol*, **1942**, 28, 285-298.

[2] J.Y. Chai, E.H. Shin, S.H. Lee, H.J. Rim, Foodborne intestinal flukes in Southeast Asia, *Korean J Parasitol*, **2009**, 47 (suppl), 69-102.

[3] J.Y. Chai, W.M. Sohn, T.S. Yong, K.S Eom, D.Y. Duk-Young Min, M.Y. Lee, H. Lim, B. Insisiengmay, B. Phommasack, H.J. Rim, *Centrocestus formosanus* (Heterophyidae): human infection and the infection source in Lao PDR, *J Parasitol*, **2013**, 99, 531-536.

[4] E.T. Han, E.H. Shin, S. Phommakorn, B. Sengvilaykham, J.L. Kim, H.J. Rim, J.Y. Chai, *Centrocestus formosanus* (Digenea: Heterophyidae) encysted in the freshwater fish, *Puntius brevis* from Lao PDR, *Korean J Parasitol*. **2008**, 46, 49-53.

[5] H.A. Pinto, A.L. Melo, Metacercariae of *Centrocestus formosanus* (Trematoda: Heterophyidae) in *Australoheros facetus* (Pisces: Cichlidae) in Brazil, *Rev Bras Parasitol Vet*, **2012**, 21, 334-337.

[6] C. Wongsawad, P. Wongsawad, K. Sukontason, W. Maneepitaksanti, N. Nantarat, Molecular Phylogenetics of *Centrocestus formosanus* (Digenea: Heterophyidae) Originated from Freshwater Fish from Chiang Mai Province, Thailand, *Korean J Parasitol*, **2017**, 55, 1, 31-37.

[7] A. Wanlop, C. Wongsawad, P. Prattapong, P. Wongsawad, T. Chontananarth, J.Y. Chai, Prevalence of *Centrocestus formosanus* Metacercariae in Ornamental Fish from Chiang Mai, Thailand,

with Molecular Approach Using ITS2, *Korean J Parasitol*, **2017**, 55, 4, 445-449.

[8] A. J. Mitchell, A. E. Goodwin, M. J. Salmon, T. M. Brandt, Experimental infection of an exotic heterophyid trematode, *Centrocestus formosanus*, in four aquaculture fishes. *N Am J Aquac*., 2002, 64, 55-59.

[9] E. Gjurčević, Z. Petrinec, Z. Kozarič, S. Kužir, K.V. Gjurčević, M. Vučemilo, P. Džaja, Metacercariae of *Centrocestus formosanus* in goldfish (*Carassius auratus* L.) imported into Croatia, *Helminthologia*, **2007**, 4, 214-216.

[10] S.M. Mood, H.A.E. Mousavi, B. Mokhayer, M. Ahmadi, M. Soltani, I. Sharifpour, *Centrocestus formosanus* metacercarial infection of four ornamental fish species imported into Iran, *Bull. Eur. Ass. Fish Pathol*, **2010**, 30, 4, 146-149.

[11] A. J. Mitchell, M. J. Salmon, D. G. Huffman, A. E. Goodwin, T.M. Brandt, Prevalence and pathogenicity of a heterophyid trematode infecting the gills of an endangered fish, the fountain darter, in two central Texas spring-fed rivers, *J Aquat Anim Health*., **2000**, 12, 283-289.

[12] R.E. Olson, J.R. Pierce, A Trematode Metacercaria Causing Gill Cartilage Proliferation in Steelhead Trout from Oregon, *J Wildl Dis*, **1997**, 33, 4, 886-890.

[13] J. Waikagul, T. Wongsaroj, P. Radomyos, V. Meesomboon, R. Praewanich, P. Jongsuksuntikul, Human infection of *Centrocestus caninus* in Thailand. *Southeast Asian J Trop Med Public Health*, **1997**, 28, 831-835.

[14] T. Chontananarth1, C. Wongsawad, Epidemiology of cercarial stage of trematodes in freshwater snails from Chiang Mai province, Thailand, *Asian Pac J Trop Biomed*, **2013**, 3, 3, 237-243.

[15] V.G.V. Paller, S. Uga, Attachment and penetration of *Centrocestus armatus* (Digenea: Heterophyidae) cercariae to gills of secondary intermediate fish hosts, *J Parasitol*, **2008**, 9, 578-583.

[16] A. J. Mitchell, R. M. Overstreet, A. E. Goodwin, T. M. Brandt, Spread of an exotic fish-gill trematode: A far-reaching and complex problem, *Fisheries*, **2005**, 30, 11-16

[17] V.S. Blazer, J.B. Gratzek, Cartilage proliferation in response to metacercarial infections of fish gills, *J Comp Pathol*., **1985**, 95, 273-280.

[18] E.M.F. Vélez-Hernández, L.J. Constantino-Casas, García-Márquez, O. Sarabia, Gill lesions in common carp, Cyprinus carpio L., in Mexico due to the metacercariae of *Centrocestus formosanus*, *J Fish Dis*., **1998**, 21, 229-232.

[19] H.A. Yildiz, Infection with metacercariae of *Centrocestus formosanus* (Trematoda:Heterophyidae) in ornamental fish imported into Turkey, *Bull. Eur. Ass. Fish Pathol*, **2005**, 25, 5, 244-246.