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Prevalence of *Haplorchoides* sp. Metacercariae and Comparative Morphology of Infected and Uninfected Scales in Cyprinoid fishes from Li River, Lamphun Province

Nareerat Chaiwang[1], Preeyaporn Butboonchoo[1, 2]* and Chalobol Wongsawad[1, 2]

[1] *Departmental of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand*
[2] *Environmental Science Research Center (ESRC), Chiang Mai University, Chiang Mai, 50200, Thailand*

*Corresponding author's e-mail address: p.bootboonchoo@gmail.com (P.Butboonchoo)

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ABSTRACT

Cyprinoid fish have been reported to have a large number of metacercariae, particularly the *Haplorchoides* metacercaria. In this study, the prevalence of *Haplorchoides* metacercaria was measured in 34 spiny barb fishes (*Mystacoleucus marginatus*) and 13 bonylip barb fishes (*Osteochilus vittatus*) collected from August 2017 to March 2018 from the Li River in the Lamphun province of Thailand. The morphology of infected scales was observed under light and scanning electron microscopes (LM and SEM). The results revealed 100% *Haplorchoides* metacercarial infection in spiny barb fishes. The highest prevalence was found in the scales of the head (55.2%), followed by the body (41.3%), and the tail (34.9%). However, the prevalence of such infection in bonylip barb fishes was observed as 69.2%, the highest rates being found in the head (27.2%), followed by the body (23.6%), and the tail (22.3%). The recovered *Haplorchoides* sp. metacercaria was rounded in shape and had 2 cyst wall layers. The larval stage within the cyst revealed that the body surface was covered with spines, a saccular-shaped excretory bladder, and three groups of acetabular spines. With LM and SEM observation, both species of uninfected fish showed cycloid-shaped scales, with a thin, smooth surface, that had no spines in the posterior part. Each scale consisted of focus, circuli, and radii on the surface. The infected metacercarial scale showed traces of metacercarial embedding that revealed a circular feature, and were thick and cracked at the ventral part of scales. The area around the metacercarial embedding was surrounded by a thick capsule.

INTRODUCTION

The trematode, *Haplorchoides,* belongs to the family Heterophyidae [1-3]. They require freshwater snails as their first intermediate host, freshwater fish as their second intermediate host, and catfish as the definitive host [4-6]. Freshwater fish, particularly cyprinoid fish, have been reported to be the second intermediate host of *Haplorchoides* spp. [6-11]. The *Haplorchoides* metacercaria is found in several parts of the fish (muscle, scale fin, and gill) but is mostly found in fish scales [7, 10]. The presence of metacercaria results in a loss of weight gain, high mortality, and low nutritive values (protein and low saturated fat) of infected fish, which is a great problem in both cultured and wild fish [12]. Additionally, a loss of scale is also present in the infected fish [13]. Harboring metacercaria in fish scale may affect the scale structure. The scale morphology of cyprinoid fish has been studied using scanning electron microscopy in many previous reports [14-17]. However, the scale structure of infected fish has not so far been studied in the northern part of Thailand.

This study aimed to investigate the prevalence of *Haplorchoides* sp. metacercaria in two cyprinoid fish species; spiny barb fishes (*Mystacoleucus marginatus*) and bonylip barb fishes (*Osteochilus vittatus*), which are found in the Li River, Lamphun province, Thailand, and are commonly consumed by humans. Also, we studied the effect of metacercarial infection on scale structure. Both uninfected and infected scale morphology was observed based on light and scanning electron microscopy (LM and SEM).

METHODOLOGY

Forty-seven cyprinoid fishes were collected from the Li River that flows through Koh Thung Man village, Ban Hong district, Lamphun province (N 18.275083, E 98.822994), Thailand. The collected cyprinoid fishes were classified into two species; *M. marginatus* (34 specimens) and *O. vittatus* (13 specimens). The scales of each fish were gently

removed with fine forceps from 3 parts, including the head (30 scales), body (30 scales), and tail (30 scales) for examination of *Haplorchoides* sp. infection under a light microscope. The number of recovered metacercaria was recorded for prevalence and intensity calculation according to Margolis et al. (1982).

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

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

Encysted and excysted metacercaria were prepared as a permanent slide for morphological study. In brief, metacercaria were washed in tap water, then fixed and flattened in 4% formalin, stained with Delafield's hematoxylin, dehydrated in an alcohol series, cleared in xylene, and permanently mounted in permount. Specimens on permanent slides were illustrated using a light microscope (OLYMPUS CHS, Olympus Optical Co., Ltd, Japan) with a drawing tube.

Both infected and uninfected scales were washed in tap water. The specimens were fixed and flattened in 4% formalin, then photographed and drawn as a figure under a light microscope (LEICA MZ6, Leica Microsystem, Germany). For SEM, the specimens were kept in 2 sandwiched slides and air dried, the dried scales were mounted on stub, gold coated, and viewed under a JEOL-JSM5400LV (Tokyo, Japan) scanning electron microscope at an accelerating voltage of 10-15 kV.

RESULTS

Forty-seven cyprinoid fishes collected from the Li River were classified into two species; M. marginatus (34 specimens) or *O. vittatus* (13 specimens). Of the 13 *O.vittatus* samples collected, 9 (69.2%) specimens were infected with *Haplorchoides* sp. metacercarial, while 100% of *M. marginatus* samples were infected (Table 1). The intensity of infection in the two fish species was 164.2 in *M. marginatus* and 252.5 in *O.vittatus* (Table 1). In both fish species, the metacercaria were found embedded in the ventral part of the head, body, and tail scale. The prevalence (Table 2) was highest in the head, followed by body, and tail scale in both *M. marginatus* and *O.vittatus*, including; head (55.2%, 27.2%), body (41.3%, 23.6%) and tail (34.9%, 22.3%), respectively. The intensity (Table 2) of infection in *M. marginatus* was highest in the head (4.7), followed by the body (4.5), and tail (2.9). *O.vittatus* showed the highest intensity in the tail (8.5), followed by the body (8.3), and head (7.2). The differences were significant among the prevalence of metacercarial infections in head, body, and tail scales in both *M. marginatus* and *O.vittatus* ($P < 0.05$).

The encysted metacercaria of *Haplorchoides* sp. (Figure A1, A2) in both *M. marginatus* and *O.vittatus* was spherical in shape and had a double layered cystic wall. The excysted metacercaria (Figure B1, B2) had a lance-shaped body, with a scale-like spine on the body surface. The oral sucker was subterminal, and the prepharynx was longer than the esophagus. The intestine extends slightly beyond the posterior part of testes. The acetabulum was submedian, with three groups of acetabular spines, including the anterior group (8-10 spines), median group (5-7 spines), and posterior group (8-10 spines) (Figure C1, C2), which are located near the intestinal bifurcation. Testes were spherical, and were located between the intestinal ends and posterior body. The ovary was round and pre-testicular. The excretory bladder was saccular and post-testicular.

LM and SEM observation, both fish species have a cycloid scale (Figure 2) covering their body. The cycloid scales have a smooth edge and no have spines on the posterior edge. The scales from different body regions revealed several different shapes. M. marginatus had a rectangle-shaped scale in the head and body regions (Figure 3A, B), while those on the tail region were pentagonal in shape (Figure 3C). Pentagonal-shaped scales were found in all regions of O. vittatus (Figure 4), but those in the tail region (Figure 4C) were longer than the head and body regions. The lateral line scales were only found in the body and tail regions, and were similar to both body and tail scales except for the presence of a lateral line canal (Figure 3C2, Figure 4C). The dorsal part of each scale was divided into 4 regions including anterior, posterior, left lateral, and right lateral fields (Figure 2A) that consisted of focus, lateral line canal, radii, circuli, and tubercle (Figure 2A and B). The focus and lateral line canal were found in the middle of the scale (Figure 5A, B, G). The lateral line canal was a long-straight longitudinal canal. The radii cross diagonally from the anterior and posterior field,

Table 1. Prevalence and intensity of *Haplorchoides* sp. metacercaria in 2 cyprinoid fish species.

Fish species	No. of examined fish	No. of infected fish	No. of metacercaria	Prevalence (%)	Intensity
M. *marginatus*	34	34	5584	100	164.2
O. *vittatus*	13	9	2273	69.2	252.5

Table 2 Prevalence and intensity of *Haplorchoides* sp. metacercaria in each part of fish scale.

Fish species	Position of scale	No. of examined scale	No. of infected scale	No. of metacercaria	Prevalence (%)	Intensity
M. marginatus	head	1020	563	2635	55.2	4.7
	body	1020	421	1900	41.3	4.5
	tail	1020	356	1049	34.9	2.9
O. vittatus	head	390	106	767	27.2	7.2
	body	390	92	765	23.6	8.3
	tail	390	87	741	22.3	8.5

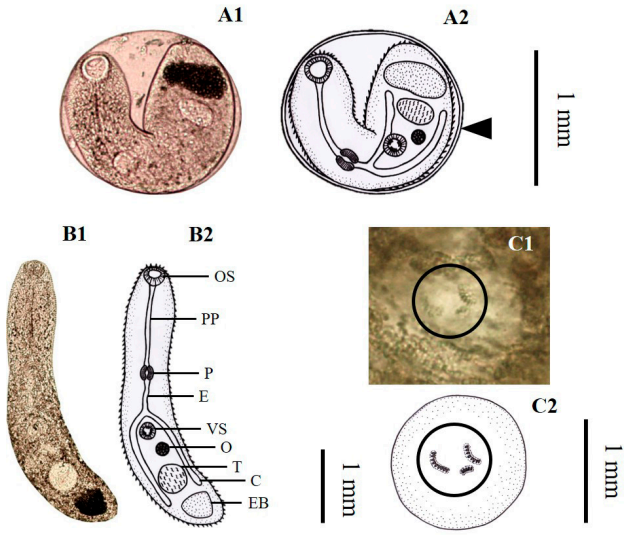

Figure 1. Morphology of *Haplorchoides* sp. metacercaria; 1: LM micrograph, 2: drawing A. Encysted metacercaria revealing spherical shape with a double layered cystic wall (arrowhead), B. Excysted metacercaria showing lance-shaped body with scale-like spine (C=ceaca, E=esophagus, EB=excretory bladder, O=ovary, OS=oral sucker, P=pharynx, PP=prepharynx, T=testes, VS=ventral sucker), C. Acetabular spines (white circle) present in three groups.

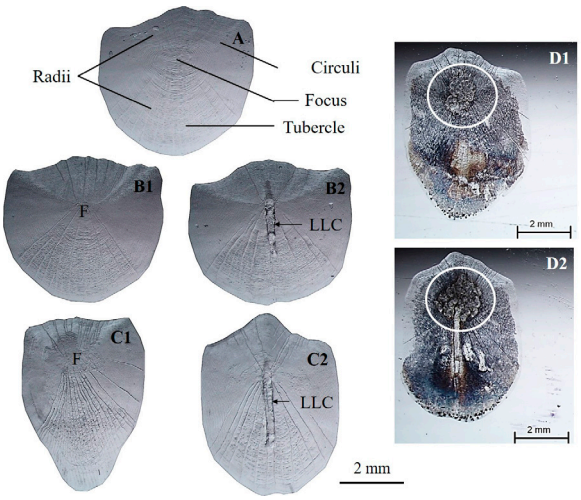

Figure 3. LM micrograph showed the different shape in head, body and tail scale of *M. marginatus*; A. Scale in head part revealed a rectangle shape, B. Scale in body part revealed a rectangular shape with a focus (F) (B1) and a lateral line canal (LLC) (B2), C. Scale in tail part revealed a pentagonal shape with a focus (F) (C1) and a lateral line canal (LC) (C2), D. Infected scale showed the thick capsule surrounding *Haplorchoides* sp. metacercaria (white circle) embedded in the anterior region of ventral part.

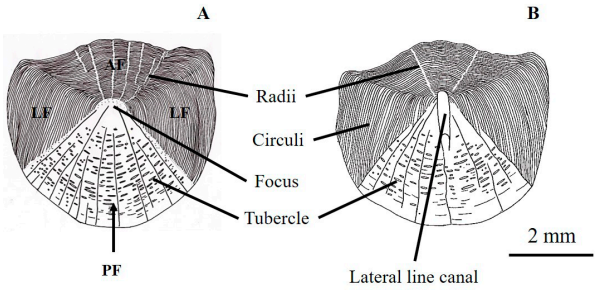

Figure 2. Scale structure of both fish species revealed cycloid scale, divided into 4 parts including; anterior field (AF), posterior field (PF), left lateral field (LF), right lateral field (LF) that consisted of radii, circuli, tubercle, focus and lateral line canal found only in the lateral line scale.

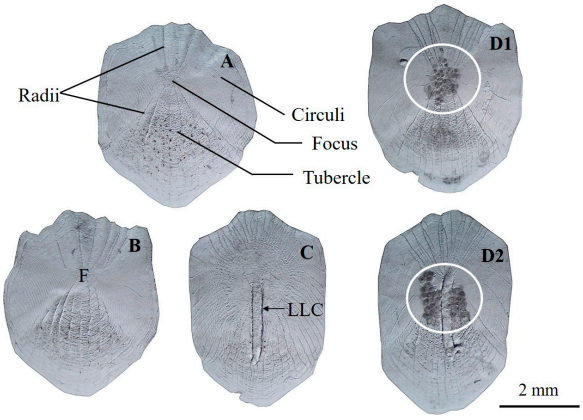

Figure 4. LM micrograph showed the similar shape in head, body and tail scale of *O. vittatus*; A, B, C. Scale in head, body and tail parts, respectively revealed a pentagonal shape with a focus (F) and lateral line canal (LLC), D. Infected scale showed the thick capsule surrounding *Haplorchoides* sp. metacercaria (white circle) embedded in the anterior region of ventral part.

and curve away from focus (Figure 5H). The circuli were defined in the anterior and lateral fields. They were arranged parallel to the edge of the fields and were found in the inter-circular space between circuli (Figure 5C, I). There were many spherical and ovoid-shaped tubercles in the posterior field (Figure 5D, J). The ventral part of the scale had a smooth surface in the uninfected scale (Figure 5E, K). The *Haplorchoides* sp. metacercaria in infected scale was found embedded in the anterior field and surrounded by a thick capsule. Some infected scales showed traces of metacercarial embedding, which presented as a circular feature. There were several cracks in the ventral part of the scale (Figure 3D, Figure 4C).

DISCUSSION

The present study found the high prevalence and intensity of the *Haplorchoides* sp.-metacercarial infection in both *Mystacoleucus marginatus* and *Osteochilus vittatus*. The prevalence of *M. marginatus* (100%) was higher than *O.vittatus* (69.2%), while its intensity was

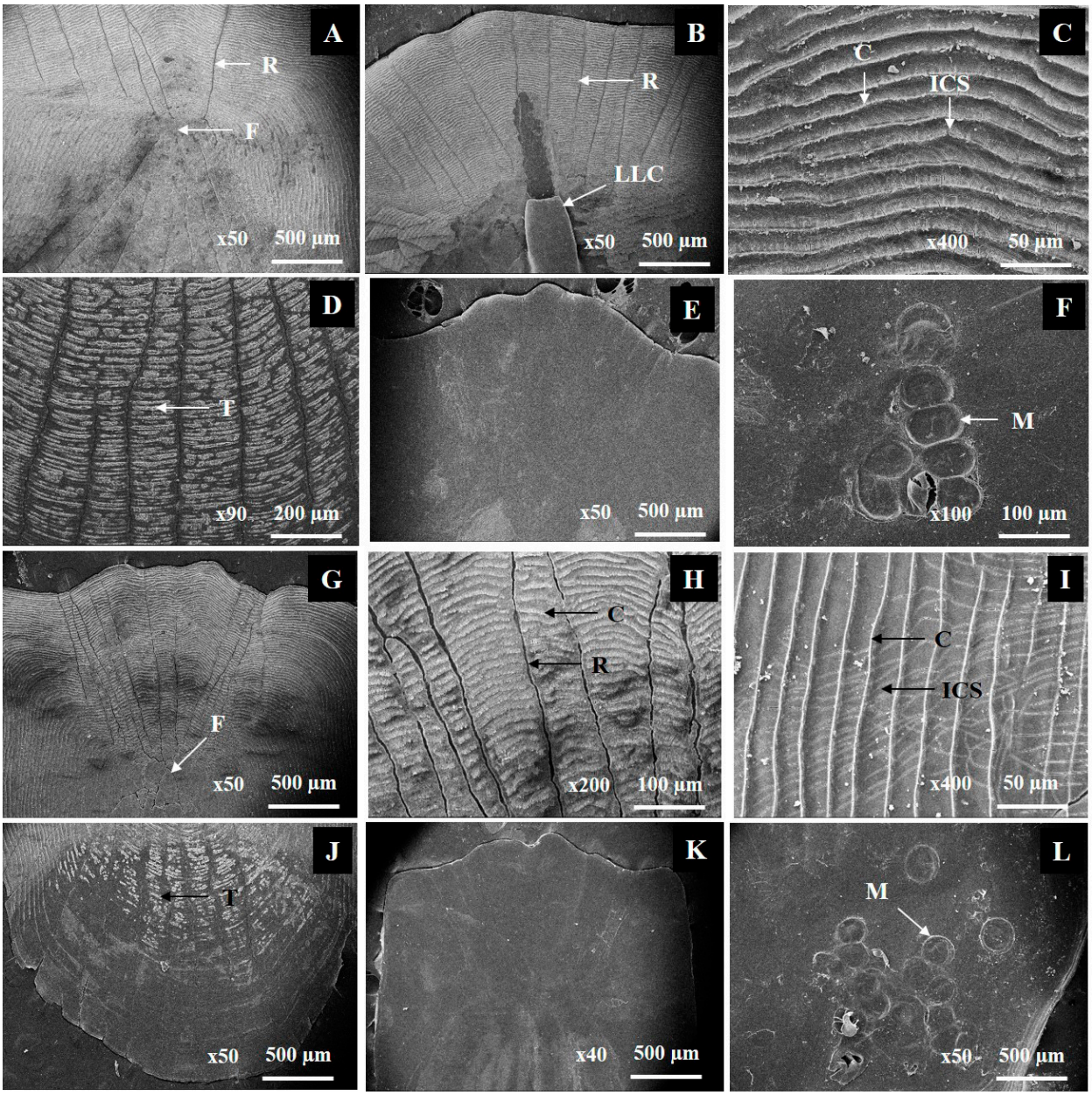

Figure 5. SEM micrograph showed the similar scale surface of *M. marginatus* (A-F) and *O. vittatus* (G-L); A, F. Dorsal part revealed a focus, radii and circuli found in the anterior region, B. Lateral line scale showed a lateral line canal, H. Radii cross diagonally from anterior field, C, I. Circuli arranged parallel to the edge of field and inter circular space between circuli, D, J. Tubercles in the posterior field, E, K. Ventral part revealed the smooth surface, F, L. Metacercarial embedding in the ventral part C= circuli, F= Focus, ICS= Inter circular space, LLC= Lateral line canal, M= Metacercaria, R= Radii, T= Tubercle.

lower. This finding agreed with that of Suntaravitun and Dokmaikaw (2014) [18], who recorded that the *Haplorchoides*-metacercarial infection of *M. marginatus* (100%) was higher than that of *O.vittatus* (26.7%). The difference of habitat affects the infection of both fish because *M. marginatus* is frequently found in lotic environments [19], whereas *O.vittatus* is found in lentic environments [20]. Due to this, *M. marginatus* has an opportunity to interact with the snail *Melanoides tubercula*, which acts as the first intermediate host. They can be infected with cercaria from the snail, which then develop into metacercaria. However, the intensity of infection in *O.vittatus* was higher than that of *M. marginatus* because they may be repeatedly infected in their habitat (lentic environment). In this study, there was a significant difference in the prevalence of metacercarial infections in the head, body, and tail scales in both *M. marginatus* and *O.vittatus* ($P < 0.05$). The infection of the scale was highest in the head scales, followed by body and tail scales in both fish. Fish use their body and tail regions for swimming [21]. The penetration and embedding of parasites in the body and tail scales are more difficult than in head scales, because both body and tail scales are always motile.

The *Haplorchoides* sp. metacercaria found in *M. marginatus* and *O.vittatus* have a similar morphology. The encysted metacercaria were spherical in shape and had a double layered cystic wall. The excysted metacercaria had a lance-shaped body, with a scale-like spine on the body surface. They have an acetabulum with acetabular spines arranged in three groups, including the anterior group (8-10 spines), median group (5-7 spines), and posterior group (8-10 spines). The number of acetabular spines is the main morphological characteristic for species identification in trematodes. This study accords with that of Apiwong et al. (2018) [11], who reported an anterior group (7-10 spines), median group (5-7 spines), and posterior group (7-10 spines) of *Haplorchoides mehrai* isolated from cyprinoid fish, *Barbonymus schwanenfeldii*, and *Cyclocheilichthys repasson* collected from Chiang Mai province in Thailand. Additionally, the shape and position of the oral sucker, prepharynx, pharynx, esophagus, caeca, testes, and ovary were similar to *H. mehrai*. The *Haplorchoides* sp. found in this study may be *H. mehrai*. However, the identification of larval stage species based on morphology alone is difficult. To remedy this, a molecular approach must be used for accurate identification in any future study.

This is the first study of scale morphology of *M. marginatus* and *O.vittatus* collected from Lamphun province, using light and scanning electron microscopes. The bodies of both species were covered by cycloid scales that have no spines on the posterior part. The result agreed with many previous studies that revealed a cycloid scale found in cyprinoid fish [14-17]. The dorsal part of the cycloid scale consisted of focus, lateral line canal, radii, circuli, and tubercle, while the ventral part had a smooth surface. There were several various scale shapes from different body regions of *M. marginatus*. Their head and body scales were rectangular in shape, but the tail scale had an elongate-pentagonal shape. The various shapes of scale in different regions depend on the position of the fish body [17]. *O.vittatus* has a pentagonal-shaped scale in all head, body, and tail regions but the tail scale was longer. The lateral line scale was only found in the body and tail regions, and its structure was similar to the body and tail scales except for the presence of a lateral line canal.

The *Haplorchoides* sp. metacercaria was found to embed in the anterior region of ventral parts of the scale. The ventral part showed a smooth surface that is easier to attach to and embed in than the dorsal part. The metacercaria was surrounded by a thick capsule because of the immunity reaction of the fish host. Some infected scales showed traces of metacercarial embedding, which present as a circular shape, and there were also cracks and tears on the scale surface. This damage was caused by the cercarial penetration and attachment by the oral sucker. The cercaria shedding from the snail host will swim to find and penetrate the fish host, then change to metacercaria [22, 23]. This study indicated that because the cycloid scale present in both studied cyprinoid fish is thin and has no spines along the scale edge, they are susceptible to *Haplochoides* sp. infection. The attachment and embedding of *Haplochoides* sp. affected the scale structure and health of both species of fish.

CONCLUSION

The present study confirmed that *Haplorchoides* sp. is prevalent in both cyprinoid fish, *M. marginatus* and *O.vittatus*, collected from the Li River in Lamphun province. This finding will also help to indicate the risk of *Haplorchoides sp.* infection in other aquatic animals. Additionally, the scale structure of the infected and uninfected scales of both fish species in northern Thailand were first studied. This suggested that the alteration of the scale surface of fish may affect their health. Due to this, freshwater fish in both natural and cultivated habitats should be protected from this parasite.

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REFERENCES

[1] H.T. Chen, Systematic consideration of some heterophyid trematodes in the subfamilies Haplorchinae and Stellantchasmi- nae, *Ann. Trop. Med. Parasitol*., **1949**, 43, 304 – 312.

[2] J.C. Pearson, C.K. OW Yang, 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(1), 35 – 60.

[3] S. Yamaguti, *Systema Helminthum Vol I. The Digenetic of Vertebrates. Part I and II*, Interscience Publisher Inc., New York, **1958**.

[4] B.P. Pande, R.P. Shukla, *Haplorchoides* Chen, 1949 (Hap- lorchinae: Heterophyidae) in freshwater fishes, *J. Helminthol*., **1976**, 50, 181 – 192.

[5] U. Shameem, R. Madhavi, The morphology, life-history and systematic position of *Haplorchoides mehrai* Pande and Shukla, 1976 (Trematoda: Heterophyidae), *J. Syst. Parasitol*., **1988**, 11, 73 – 83.

[6] T. Scholz, O. Ditrich, M. Giboda, Differential diagnosis of opisthorchiid and heterophyid metacercariae (Trematoda) infecting flesh of cyprinid fish from Nam Ngum Dam Lake in Laos, *Southeast Asian J. Trop. Med. Public. Health*., **1991**, 22, 171 – 173.

[7] S. Saenphet, C. Wongsawad, K. Saenphet, A survey of Helminths in freshwater animals from some areas in Chiang Mai, *Southeast Asian J. Trop. Med. Public. Health*., **2001**, 32(2), 2102 – 2113.

[8] C. Nithikathkul, C. Wongsawad, Prevalence of *Haplorchis taichui* and *Haplorchoides* sp. metacercariae in freshwater fish from water reservoirs, Chiang Mai, Thailand, *Korean J. Parasitol*., **2008**, 46(2), 109 – 112.

[9] W. Noikong, C. Wongsawad, A. Phalee, Seasonal variation of metacercariae in cyprinoid fish from Kwae Noi Bamroongdan Dam, Phitsanulok Province, northern Thailand, *Southeast Asian J. Trop. Med. Public. Health*., **2011**, 42(1), 58 – 62.

[10] Y. Manpratum, W. Kaewkes, P. Echubard, B. Sripa, S. Kaewkes, New locality record for *Haplorchoides mehrai* and possible interactions with *Opisthorchis viverrini* metacercariae in cyprinid fishes in Northeast Thailand, *Parasitol. Res*., **2017**, 116, 601 – 608.

[11] K. Apiwong, C. Wongsawad, P. Butboonchoo, Morphological and molecular characterization of *Haplorchoides mehrai* Pande and Shukla 1976 (Digenea: Heterophyidae) from Chiang Mai province, *Helminthologia*, **2018**, 55(4), 334-342.

[12] G.A.K. Kirrella, N.M. Elhawary, E.K. Bazh, S.S.Gh. Sorour, K.M. El-Dakhly, *Oreochromis niloticus* and *Clarias gariepinus* Fish Infected with Digenean Metacercariae: Experimental Infection of Puppies and Some Chemical Alterations in Fish, *Asian J. Anim. Vet. Adv*., **2018**, 13(4), 352-359.

[13] S. Aly, I. Eissa, A. Badran, M. Elamie, B. Hussain, Pathological Studies on Encysted Metacercariae Infections among some Freshwater Fish in Egyptian Aquaculture, **2005**

[14] L.A. Jawad, Comparative morphology of scales of four teleost fishes from Sudan and Yemen. *J Nat. Hist.*, **2005**. 39(28), 2643-2660.

[15] H.R. Esmaeili, Z. Gholami, Scanning electron microscopy of scales in cyprinid fish, *Alburnoides bipunctatus* (Blotch, 1782), *Iran J Fish Sci*, **2008**, 10(1), 155-166.

[16] H.R. Esmaeili, A. Gholamifard, N. Zarei, A. Arshadi, Scale structure of a cyprinid fish, *Garra Rossica* (Nikol'skii, 1900) using scanning electron microscope (SEM). *Iran J Sci. Tech*., **2012**, A4, 487-492.

[17] M.A.M. Ganzon, M.A.J. Torres, J.J. Gorospe, C.G. Demayo, Variations in Scale Morphology between Sexes of the Spotted Barb, *Puntius Binotatus* (Valenciennes, 1842) (Actinopterygii: Cyprinidae), International Conference on Environment and Bio Science, **2012**, IPCBEE Vol. 44.

[18] P. Suntaravitun, Ng. Dokmaikaw, Prevalence of Trematode Metacercariae in Cyprinoid Fish from Mae Lao District in Chiang Rai Province, *KKU Sci. J.*, **2014**, 42(3), 544-550.

[19] T.R. Roberts, The freshwater fishes of Western Borneo (Kalimantan Barat, Indonesia, *Mem. Calif. Acad. Sci*, **1989**, 14, 210.

[20] K.P. Lim, K.L. Ng, *A Guide to the Freshwater Fishes of Singapore*. Singapore Science Centre, **1990**.

[21] S.G. Duran, D. Arola, E.A. Oss, Effect of chemical composition and microstructure on the mechanical behavior of fish scales from Megalops Atlanticus, *J Mech Behav Biomed Mater*., **2016**, 56, 134–145.

[22] 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**, 94(3), 578-583.

[23] C. Donthaisong, P. Arunsan, K. Suwannatrai, S. Prasopdee, J. Kulsantiwong, S. Wongmaneeprateep, A. Suwannatrai, S. Tesana, Infectivity and development of Opisthorchis viverrini metacercariae inimmuno suppressed *Barbonymus gonionotus* fingerlings (Cyprinidae), *Acta Tropica*, **2016**, 162, 107–113.