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Zoonotic Fish Parasites in Riau, Indonesia

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ABSTRACT

Clinostomum sp. is belonged to digenetic trematode that commonly infests freshwater fishes. This parasite is zoonotic and may cause *laryngopharyngitis* disease in human that consume raw fish. A study aims to understand the morphology and prevalence of *Clinostomum* sp. in the freshwater fishes from Riau, Indonesia has been conducted from February to October 2013. Fish samples were measured and weight and then were investigated for identifying the parasites infection. Parasites were taken manually and fixed in 10% formaline solution and stained with *Semichon's acetocarmine*. Parasites were then studied using a stereo microscope, the number and types of parasites were noted and examined. Morphological characteristics of the cysts are ellipse, 0,03 (0,02-0,04 mm) length and 0,02 (0,01-0,03) mm width, transparent, off white colored and attach in the fish tissue. Based on its morphological characteristics, *Clinostomum* sp. found were identified as *C. phalacrocoraxis*. Results of this research prove that *T. trichogaster* is the 2nd intermediate host of *Clinostomum* sp in Riau Province, Indonesia.

Keywords: *Metacercariae*, *Clinostomum*, fish parasite, Riau

INTRODUCTION

Freshwater fish are an important source of protein for people in various areas, but sometimes the fishes were infected with parasites. Zoonotic fish parasites are transmitted by fish and fish products, and pose a major public health problem. People become infected with zoonotic parasites after ingesting raw or undercooked freshwater fish containing infective metacercariae. Nowadays a total of 59 fish-borne parasitic zoonoses species, which are known to parasitize humans, are listed. All species can be divided into two groups, the first being the small liver flukes (Opisthorchiidae: 12 species), and the second the minute intestinal flukes (Heterophyidae: 36 species, Echinostomatidae: 10 species and Nanophyetidae: 1 species) (Murrell and Fried, 2007). Adults of the small liver flukes parasitize the liver (bile ducts and gall bladder) of their definitive host, where they can cause serious diseases in humans. Cholangitis, choledocholithiasis, pancreatitis, and cholangiocarcinoma are the major clinical problems associated with chronic infections. Individuals with light infections usually show no symptoms. Intestinal flukes are generally not of considerable clinical importance compared to the liver flukes, but several species may cause significant pathology sometimes fatal; in the heart, brain, and spinal cord of humans (Hung *et al.*, 2015).

There are a moderate number of nematodes, trematodes, cestodes and acanthocephalans which have been reported in humans, but only a few cause serious disease. All of the worms, however, are associated with social-cultural and behavioural factors which enhance infection, especially the habit of eating raw fish. For examples of common raw seafood dishes known to transmit parasitic zoonoses are Japanese sushi (raw seafood surrounded with rice and nori wrap), Japanese sashimi (thinly sliced rawfishes), Japanese salad (raw fish, fresh lettuce, and soya sauce) (Adam *et al.*, 1997).

In Indonesia, eating raw fish dishes (e.g sushi and sashimi) can be found in the Chinese Restaurant or in tradisional Batak dish derived from Tapanuli (known as *Na-Niura* means that "the fish is not cooked"). Eating raw fish dishes is popular in some areas in Indonesia and causing the spread of fish borne-parasites that can infect humans.

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The most important zoonotic fish parasites in freshwater fish are *Heterophyes heterophyes*, *Metagonimus yokogawai* and *Clinostomum* sp. These parasites, acquired by eating raw, marinated or improperly cooked fish, are frequently reported from human infections in the Middle East and Asia, especially the Philippines, Indonesia, Thailand, the People's Republic of China, Japan and the Republic of Korea. The accumulation of large numbers of these parasites in the small intestine may cause inflammation, ulceration and necrosis (Williams and Jones, 1994).

The Clinostomidae Luhe, 1901 is a family of digeneans the members of which, at the adult stage, live in the oral cavity, pharynx or oesophagus of fish, reptiles and occasionally mammals, including man (Gustinelli *et al.*, 2010). One type of parasites that is able to infect human being is the zoonotic parasite, *Clinostomum* sp. that causes laryngopharyngitis disease. The laryngopharyngitis cases have been found in many Asean countries such as Thailand, Philippines, China and Japan. The human laryngopharyngitis case was reported in Korea (Chung *et al.*, 1995), Iran and Japan (Kifune *et al.*, 2000), but there is no reported case in Indonesia. However, this case may occur in Indonesia as there is infection of *Clinostomum* in edible freshwater fishes in Indonesia.

Clinostomum sp. belonged to digenetic trematode and commonly found in the throat and oesophagus of Piscivorous birds (Yamaguti, 1958). *Clinostomum* metacercariae, known as "yellow grubs" due to the colour of their gut contents, may encyst in different sites (e.g. dermis, muscles, gill-arch) or remain free in the body-cavity. The parasite's cysts present in the improper cooked or raw fish has potential to distribute the parasite to other type of host. Pica *et al.*, 2003 said that in Thailand, *Clinostomum* sp. metacercariae in the *Trichogaster trichopterus* that has been fermented (known as Plah-ra) or salted fish is survive for 7 days.

Many types of freshwater fish has been infected by *Clinostomum*. The fishes represent as a 2nd intermediate host of the parasite. The parasite has been found in *Carassius carassius* (Chung *et al.*, 1995), *Cyprinus carpio* (Aohagi *et al.*, 1992), *Oreochromis niloticus* and *Cobitis anguilla caudatus* (Dias *et al.*, 2006). The average of *C. complanatum* prevalence in *Oreochromis niloticus* in the Saudi Arabian is 50.2% (Abo Essa, 2000). In Indonesia *Clinostomum* infestation has been found in the gouramy fish cultured in paddy fields in Purwokerto, Central of Java (Kabata *in* Handajani, 2005). There are many researches prove that the Siak River is located in Riau Province is polluted. This river has a bad water quality and fish species was living in that river is infected with parasites and in danger. Around 2 decades ago, many fish species, including the high economical valued edible fishes and ornamental fishes were present in the Siak River (Windarti, 2014). However, there is no information on morphological characteristics of metacercariae of the parasite and the occurrence of this species. To get information on the morphological characteristics of *Clinostomum* as zoonotic fish parasites in Riau, Indonesia, a study is needed.

MATERIALS AND METHODS

This study has been conducted from February to October 2013. There are 16 freshwater fishes were collected from the Sail River, Riau Province. The fishes were captured using gill nets, weight, measured and identified based on Saanin (1987). The fishes were identified and examined for helminths. Each fish was separated into 6 parts: fin rays (all the fins: dorsal, pectoral, pelvic, anal, and caudal), scales, gills, muscles, intestine, thorax and abdominal cavities. The parasites were observed under a stereomicroscope and the numbers were recorded. Digenea, encysted metacercariae of *Clinostomum* were excysted using a needle. The occurrence of *Clinostomum* sp. cysts in the body of the fish was investigated macroscopically.

The cysts were then opened, the metacercariae were fixed with 10% formaline solution and was stained using *Semichon's acetocarmine* (Pritchard and Kruse, 1982). Some helminths were drawn by drawing tube. Helminth classification was based on Yamaguti (1958); Velasquez, 1975; Hanafi (1983); Chung *et al.*,(1995).

RESULTS AND DISCUSSIONS

There were 16 fish species captured in the Sail River, Riau Province namely *Cyprinus carpio*, *Clarias batrachus*, *Oreochromis niloticus*, *Channa striata*, *Helostoma temminchi*, *Pristolepis Grooti*, *Mystus numerus*, *Channa micropeltes*, *Osteochilus kahajanensis*, *Labiobarbus ocellatus*, *Puntius bulu*, *Kryptopterus apogon*, *Anabas testudineus*, *Kryptopterus kryptopterus*, *Rasbora argyrotaenia*, *Trichogaster trichopterus* (Table 1).

Among the 16 fishes captured, however, one species of trematode, *Clinostomum sp.* (metacercariae) was found in the gills of *Trichogaster trichopterus* only. It is the first record of the *Clinostomum* infestation in this type of fish and this fish represent as 2nd intermediate host of *Clinostomum sp.* in Sumatera, Indonesia. Although 33 species of digenetic trematodes have been listed as transmissible to man through the consumption of fish, crustacea or molluscs, only a few represent notable zoonotic threats. Among these, the members of the Clinostomidae family are significant. This group comprises very small trematodes which inhabit the intestine of birds and mammals. The infective stage (metacercariae) can be found in a widevariety of fresh and marine fish (Sprent, 1969).

Camallanus anabantis infects many species of freshwater fish(Pearse, 1933; Kumchoo *et al.*,1999; Wongsawad *et al.*, 2004).One metacercariae of *Clinostomum philippinensis* was found in the gills of *Trichogaster microlepis* (Yooyen *et al.*, 2006). In the Philippines, *Clinostomum philippinensis* Valasquez, 1959 was thefirst record of this species in *Ophiocephalus striatus*. The worm encysted in the tissues outside the eyeball and linings of the pericardial and opercular cavities and tissues under the pectoral fins of fish (Velasquez,1975).

Other records of *C. complanatum* have been reported by Hanafi (1983) that found the parasite in the visceral organ of *Trichogaster trichopterus* (Pallas) from fresh water in Ayutthaya Province,India. Yooyen *et al.*, (2006) found *C. philippinensis* in the intestine of *T. microlepis* in Thailand. Lo *et al.*, (1987) stated that there are 3 freshwater fish species that represent as 2nd intermediate host of *Clinostomum sp.*, they are *Acheilognathus koreensis*, *Rhodeus uyeki*and *Squalidus gracilis majimae*. Aohagi *et al.*,(1992) states that other freshwater species such as *Clarias batrachus* and *Cyprinus carpio*also represent as 2ndintermediate host of *Clinostomum sp.*

The prevalence of *Clinostomum sp.* metacercariae in the males (76.2%) is higher than that of the females (23.8%). Based on fish size, the lowest metacercariae prevalence (2.9%) is in the fish with at least 13.8 cm TL, while the highest (39.1%) is in fish with 9.3-11.5 cm TL. Body weight range of the infected fish is arround 2,99 – 14,98 g (males). 3.40-14.35 g (females). Results in this research are similar to results obtained by Malek and Mobedi (2001) stated that the prevalence of *C. complanatum* metacercariae in female *Channa punctatus* (50%) is higher than that of the males (33%). In contrast to Kalantan *et al.*, (1985), who stated that *C. complanatum* metacercariae infection in the female *Aphanius dispar* is 47.5% and it is lower than that of the males (55.9%). These data indicate the infection of *Clinostomum sp.*is not only depend on the size of the fish but alsothe sexuality of the fish.

Metacercariae of *C. complanatum* is able to infest any body part of the fish. In *T. trichopterus*, the cysts are found in the thorax and abdominal cavities (1-29 cysts/ fish). The number of cyst in the *T. trichopterus* is lower than the cysts found in Cyprinids, that are range from 1 to 60 cysts/ fish (Malek and Mobedi, 2001) and in *Anabas testudineus* from Yogyakarta, Indonesia, which is 1-2 cysts/ fish (Riauwaty, 2011).

Table 1. Distribution of helminths in freshwater fish from Riau Province, Indonesia

Fish species	Numberd of fish infected/ examined	Helminths species	Number of helminths in each part						Total
			Fins	Mus cle	Gills	Abdo minal cavity	Intes tine	Thorax cavity	
<i>C.carpio</i>	1/50		0	0	0	0	0	0	0
<i>Clarias batrachus</i>	10/50	<i>Pallisentis</i> sp.	0	0	0	0	10	0	10
<i>Oreochromis niloticus</i>	0/50		0	0	0	0	0	0	0
<i>Channa striata</i>	5/50	<i>Pallisentis</i> sp.	0	0	0	0	5	0	5
<i>Helostoma temminchi</i>	0/50		0	0	0	0	0	0	0
<i>Anabas testudineus</i>	1/50	<i>Pallisentis</i> sp.	0	0	0	0	1	0	1
<i>Pristolepis Grooti</i>	0/50		0	0	0		0	0	0
<i>Mystus numerus</i>	2/50	<i>Cammalanus anabantis</i>	0	0	0	0	2	0	2
<i>Channa micropeltes</i> ,	8/50	<i>Cammalanus anabantis</i>	0	0	0	0	8	0	8
<i>Osteochilus kahajanensis</i>	0/50		0	0	0	0	0	0	0
<i>Labiobarbus ocellatus</i>	0/50		0	0	0	0	0	0	0
<i>Puntius bulu</i>	0/50		0	0	0	0	0	0	0
<i>Kryptopterus apogon</i>	0/50		0	0	0	0	0	0	0
<i>Kryptopterus kryptopterus</i>	0/50		0	0	0	0	0	0	0
<i>Rasbora argyrotaenia</i>	0/50		0	0	0	0	0	0	0
<i>Trichogaster trichopterus</i>	18/105	<i>Clinostomum</i> sp.				10	0	8	18

Occurance of *Clinostomum* sp. Metacercariae in *Trichogaster trichopterus*. The occurrence of *Clinostomum* sp. metacercariae in the *T. trichopterus* captured in the Sail River, Riau Province. Among 105 fish captured, 18fishes were infected by *Clinostomum* sp.metacercariae (the prevalence was 17.4%). The prevalence of metacercariae in the thorax cavity is 15.3% and the other is present in the abdominal cavity (65.7%).

Table 1. The prevalence of *Clinostomum* sp.metacercariae in the *T. trichopterus* originated from the Sail River, Riau Province

Parasite infection	Prevalence of parasite infestation (%)
Thorax cavity (n=16)	15.3
Abdominal cavity (n=69)	65.7
Male (n=80)	76.2
Female (n=25)	23.8
Fish length (cm)	
≤ 7 (n=23)	21.9
7.1-9.2 (n=29)	27.6
9.3-11.5 (n=41)	39.1
11.6-13.7 (n=29)	27.6
≥ 13.8 (n=3)	2.9
Total (n=105)	17.4

In this study, *Clinostomum* sp. infection cases in the small fish is higher than that of the bigger fish. According to Malek and Mobedi (2001), smaller fish may more vulnerable to *C. complanatum* infection, as the parasite immunity of the big fish is higher than that of the small fish. As a

consequence, the parasite prevalence is significantly decrease as the fish growing. Kabata (1985) stated that *Clinostomum* sp. infection in gouramy (*Osphronemus gouramy*) fingerlings (2-3 cm) has been found in Purwokerto, Central of Java. Lo *et al.*, (1981) also found that *C. complanatum* infect 8% of *Carassius auratus* fingerling in Japan and this parasite also infects *Plecoglossus altvelis* in Taiwan.

Description of Helminths *Clinostomum* sp. (Metacercariae). The parasite's body is linguliform. *Clinostomum* metacercariae cysts are distributed in the thorax and abdominal cavities of *T. trichopterus* (Figure 1). The cysts are ellipse, 0,03 mm (0,02-0,04 mm) length and 0,02 mm (0,01-0,03) mm width, transparent, off white and attach in the fish tissue. *Clinostomum* metacercariae may present in many body parts of fish. Aohagiet *al.*,(1992) stated that metacercariae of *Clinostomum* sp. present in the intestine of *Oreochromis niloticus* and *Sarotherodon galilaeus* captured in the Niger River, Kenya, while Dias *et al.*, (2003) obtained the metacercariae in the muscle and visceral organs of several freshwater fish species in Brazil.

By using a *semichon's acetocarmin* staining method, the morphological characteristics of the *Clinostomum* metacercariae can be seen clearly. The metacercariae is tongue-shape, relatively short (3.47-3.27 mm length and 0.84-1.45 mm width). No integument spine. Oral sucker small, anteriorly sub-terminal and slightly oval (0.14-0.18 mm length and 0.17-0.28 mm width). Ventral sucker close to oral sucker, bigger than oral sucker, in the anterior 3rd of the body. Distance between oral sucker and ventral sucker is 20.7 (20-21.4) mm. Caeca wide and very close to oral sucker. The caeca is 327,2 (261,5-392,9) mm length and 97,2 (5,7-8,6) mm width. The testes are slightly lobed and located in the posterior part of the body. Uterus elongated, extended from intertesticular to post acetabular region. The ovary is oval and small, located between testes (Figure 1). Based on Chung *et al.*, 1995, the *Clinostomum* sp. from Riau is identified as *Clinostomum phalacrocorasis*.

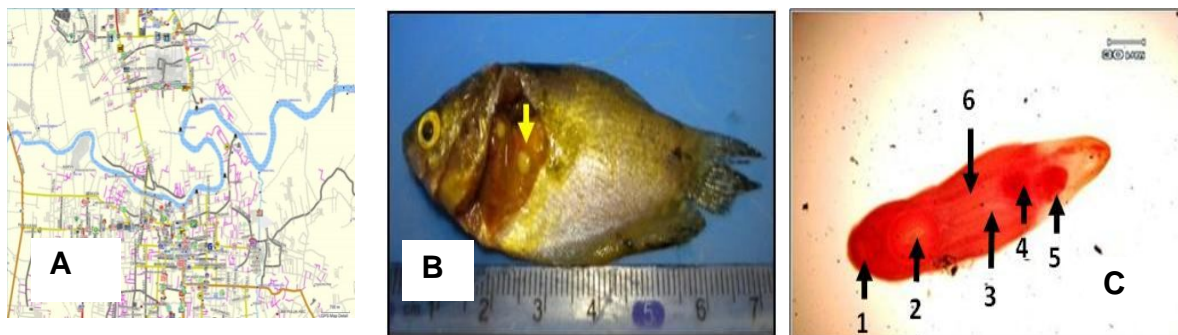


Figure 1. B. Metacercariae of *Clinostomum* sp. (Scale bar 0.1 mm). C. Ventral view, 1. Oral sucker, 2. Ventral sucker, 3. Uterus, 4. Anterior testes, 5. Posterior testes, 6. Caeca.

Usually, freshwater fish in the genus *Trichogaster* are cooked in the form of salted fish, a traditional Indonesia dish in some areas. When people are eating raw and/or undercooked fish is believed to be the source of metacercarial infections of humans. Heat inactivation of parasites is the single, most effective method for eliminating the risk of parasitic infections and can be achieved during processing or by the consumer. However, parasites, such as worms, will still be present within the product and will be visible to the consumer. Time/temperature dependent after a minimum temperature is attained. For conventional cooking (including baking), the internal temperature of the thickest part of the product should reach a minimum of 63°C (145°F) for 15 seconds or longer. Cooking with a microwave oven requires a higher temperature to kill all the parasites due to the

uneven heating which occurs: a temperature of 77°C (170°F) in the thickest part of the product is recommended (Miller *et al.*, 1994).

CONCLUSION

Based on data obtained, it can be concluded that themorphological characteristics of *Clinostomum* sp. found in *Trichopterus trichogaster* is linguliform, the cysts are ellipse, 0,03 mm (0,02-0,04 mm) length and 0,02 mm (0,01-0,03) mm width, transparent, off white and attach in the fish tissue. Results of this research prove that *T. trichogaster* is a new record and this fish represent as the 2nd intermediate host of *Clinostomum* sp. in Riau Province, Indonesia.

REFERENCES

- Abo Essa, F.J. 2000. Role of fish eating bird *Ibis ibis* in transmitting some parasites larval stages to freshwater *Oreochromis niloticus* fish. Egypt. *J. Agric. Res.* 78 (1): 105-116.
- Adams A.M. & Rausch R.L. (1997). Diphyllbothriasis. In Pathology of infectious diseases, Vol. 2 (D.H. Connor, F.W. Chandler, D.A. Schwartz, H.J. Manz & E.E. Lack, eds). Appleton and Lange, Stamford, 1377-1389.
- Aohagi Y, Shibahara T, Machid N, Yamaga Y, Kagota K. 1992. *Clinostomum complanatum* (Trematoda: Clinostomidae) in Five New Fish Hosts in Japan. *J. Wildl. Dis.* 28 (3): 467-469.
- Bush A.O, Lafferty K.D, Lotz J.M, Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al.*, revisites. *J. Parasitol.* 83 (4): 575-583.
- Chung D, Kong H.H, Joo C.Y. 1995. Demonstration of the second intermediate hosts of *Clinostomum complanatum* in Korea. *The Korean J. Parasitol.* 33 (4): 305-312.
- Dias M.L.G.G, Eiras J.C, Machado M.H, Souza G.T.R, Pavanelli G.C. 2003. The life cycle of *Clinostomum complanatum* Rudolphi, 1819. (Digenea, Clinostomidae) on the floodplain of the High Parana River, Brazil. *Parasitol. Res.* 89: 506-508.
- Dias M.L.G.G, Minte-Vera C.V, Eiras J.C, Machado M.H, Souza G.T.R, Pavanelli G.C. 2006. Ecology of *Clinostomum complanatum* Rudolphi, 1814 (Trematoda: Clinostomidae) infecting fish from the floodplain of the high Parana River, Brazil. *Parasitol. Res.* 99: 675-681.
- Handajani H, Samsundari S. 2005. Parasit dan Penyakit Ikan. Universitas Muhammadiyah Malang. 201.
- Hanafi K. 1983. Studies on the helminth parasites infected in Pla-Kadi-Mor *Trichogaster trichopterus* (Pallas) from natural water at Mueang District, Ayuthaya Province. Thesis. Kasetsart University, 1983: 133.
- Hung, N.M, Dung, D.T, Lan Anh, N.T, Van, V.T, Thanh, B.G, Van Ha, N, Hien, H, Canh, L.X, 2015. Current status of fish-borne zoonotic trematode infections in Gia Vien district, Ninh Binh province, Vietnam. *Parasites & Vectors* 2015, 8:21 pp 11186/s13071.
- Kabata Z. 1985. Parasites and Diseases of Fish Culture in the Tropics. Philadelphia, Taylor & Francis Limited. 21.
- Kalantan A.M.N, Arifin M, Nizami W.A. 1985. Seasonal incidence and pathogenicity of the metacercariae of *Clinostomum complanatum* in *Aphanius dispar*. *Japanese J. Parasitol.* 36: 17-23.
- Kifune T, Ogata M, Miyahara M. 2000. The first case of Human Infection with *Clinostomum* (Trematoda: Clinostomidae) in Yamaguchi Prefecture, Japan. *Med. Bull. Fukuoka Univ.* 27 (2): 101-105.
- Kumchoo K, Wongsawad C, Sirikanchana P, Sripalwit P. Light microscopy and scanning electron microscopy of *Camallanus anabantis*, Pearse, 1933 (Nematoda: Camallanidae) from *Anabastestudineus* Bloch, 1972. *Songklanakarin J. Sci. Technol.* 1999;23:185-91.
- Lo C.F, Kou G.H, Huber F, Liu F.G. 1987. The study of *Clinostomum complanatum* Rudolphi 1819. The metacercariae of *Clinostomum complanatum* in the sweet fish (*Plecoglossus altivelis*). The memoir of parasitology in fish disease, 2 (11): 56-63.
- Lo C.F, Huber F, Kou G.H, Lo C.J. 1981. Studies of *Clinostomum complanatum* (Rudolphi 1819). *Fish Pathol.* 15: 219-227.
- Malek M, Mobedi I. 2001. Occurrence of *Clinostomum complanatum* (Rudolphi, 1819) (Digenea: Clinostomidae) in *Capoeta capoeta gracilis* (Osteichthys: Cyprinidae) from Shiroud River, Iran. *Iranian J. Publ. Health.* 30: 95-98.
- Miller K.S., Adams A.M., Wekell M.M. & Dong F.M. (1994).- Survival of *Anisakis simplex* in microwave-processed arrowtooth flounder (*Atheresthes stomias*). International Food Technologists Annual Meeting, 25-29 June, Atlanta, Georgia. Institute of Food Technologists, Chicago, Illinois, 162.
- Pearse AS. Parasites of Siamese fishes and crustaceans. *J. Siam Soc (Nat Hist Suppl)* 1933;9:179-91.
- Prichard M.H, Kruse G. 1982. The collection and preservation of animal parasites. Lincoln, NE: University of Nebraska Press. 147.
- Riauwaty, M. 2011. Studi perbandingan morfologi dan molekuler *Clinostomum complanatum* (Digenea: Clinostomidae) pada ikan air tawar di Yogyakarta dan Riau. Disertasi. Universitas Gadjah Mada. 140 halaman.
- Saanin H. 1984. Taksonomi dan Kunci Identifikasi Ikan. Jilid I dan II. Cetakan ketiga. Bina Cipta. Jakarta. 205.
- Sprent J.F.A. (1969). Helminth zoonoses: an analysis. *Helminthol. Abstr.*, 38 (3), 333-351.
- Velasquez CC. Digenetic trematodes of Philippine fishes. Quezon City: The University of the Philippines Press, 1975.

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- Windarti. 2014. A study on Otolith of living in the Siak River. Proceeding of the 3rd International Seminar of Fisheries and Marine Science. Pekanbaru. pp 1-9.
- Yamaguti S. 1958. Studies on the helminth fauna of Japan. Part I. Trematodes of birds, reptiles and mammals. *Japanese J. Zool.* 5: 66-71.
- Yooyen. T, Wongsawad, C.Kunchoo, K, Chaiyapo. M. 2006. A new record of *Clinostomum philippinensis* (Valasquez,1959) in *Trichogaster microlepis* (Günther, 1861) from Bung Borapet, Nakhon sawan, Thailand. *Southeast Asian J. Trop. Med. Public Health* 100 (37) (suppl 3).