

## ***Euclinostomum heterostomum* (Rudolphi, 1809) Metacercarial Infection in Three Osphronemid Fish Species**

**Watchariya PURIVIROJKUL<sup>1,\*</sup> and Montri SUMONTHA<sup>2</sup>**

<sup>1</sup>*Animal Systematics and Ecology Speciality Research Unit, Department of Zoology, Faculty of Science, Kasetsart University, Chatuchak Bangkok 10900, Thailand*

<sup>2</sup>*Ranong Marine Fisheries Station, Paknam, Muang, Ranong 85000, Thailand*

(\* Corresponding author's e-mail: fsciwyp@ku.ac.th)

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### **Abstract**

Trematode metacercariae, *Euclinostomum heterostomum* from naturally infested osphronemid fish in Thailand were investigated. Encysted musculature was observed in the case of heavy infestation in all three osphronemid fish species, *Trichopsis vittata*, *Betta splendens* and *Betta imbellis*. Infected fish showed tubercle-like thickened areas of different size, 0.2 - 0.5 cm which appear on the skin. A histological study showed metacercaria were enclosed by a thin sheet of connective tissue. This is a new recorded host of *E. heterostomum*.

**Keywords:** *Euclinostomum heterostomum*, metacercaria, osphronemid fishes, Thailand

### **Introduction**

Many species of freshwater fish serve as a second intermediate host of the digenetic trematode. Thus far only five families of digenetic trematode metacercariae have been reported from freshwater fishes, viz., Clinostomidae, Diplostomidae, Isoparorchidae, Strigeidae and Heterophyidae [1]. Encysting trematode metacercariae were found in different organs of the fish such as fins, skin, gills, branchial chambers, body cavities, kidneys and other internal organs [2]. Some species of clinostomid metacercariae were reported for *Trichogaster* spp. (Osphronemidae), metacercaria of *Clinostomum piscidium* Rudolphi was found for *Trichogaster fasciata* Bloch and Schneider in India [3]. *Clinostomum philippinensis* Velasquez was found for *Trichopodus microlepis* (Gunther) in Thailand [4]. In this survey, three species of osphronemid fish, *Trichopsis vittata* (Cuvier), *Betta splendens* Regan and *Betta imbellis* Ladiges were examined for those infected by clinostomid metacercariae. This study aimed to identify the parasite, record clinical signs of the infection and analyze the histopathology related to the infection.

### **Materials and methods**

Three species of osphronemid fishes, *Trichopsis vittata*, *Betta splendens* and *Betta imbellis* were collected from Ranong province and Phuket province (Southern Thailand). In Kra-buri district of Ranong province, two species of osphronemid fishes, *T. vittata* and *B. splendens* were found to be infected with metacercariae. In Talang district of Phuket province, two species of osphronemid fishes, *B. imbellis* and *T. vittata* were found to be infected with metacercariae. Collected specimens were fixed in 70 % ethanol, stained with Semichon's acetic acid and carmine, dehydrated, cleaned in xylene and mounted in Canada balsam for further morphological examination. The existing taxonomic keys [5-8] were used for proper identification. Infected muscles were fixed by 10 % buffered formalin, dehydrated through a graded ethanol series and embedded in paraffin for histopathological study. Samples were cut into six-micrometer-thick sections and stained with hematoxylin and eosin (H&E).

## Results and discussion

Trematode metacercariae were found in the muscular tissues of three wild species of osphronemid fishes, *T. vittata*, *B. splendens* and *B. imbellis* from Southern Thailand. These metacercariae were not found in another fish family in the same aquatic habitat. Parasitic intensities varied from 1 - 16 metacercarial cysts per infected fish (**Figure 1A**). The cyst diameter ranged from 2 to 5 mm. After scale removal, a group of encysted metacercaria was observed, in some areas the group of metacercariae consisted of 6 metacercariae (**Figure 1B**).

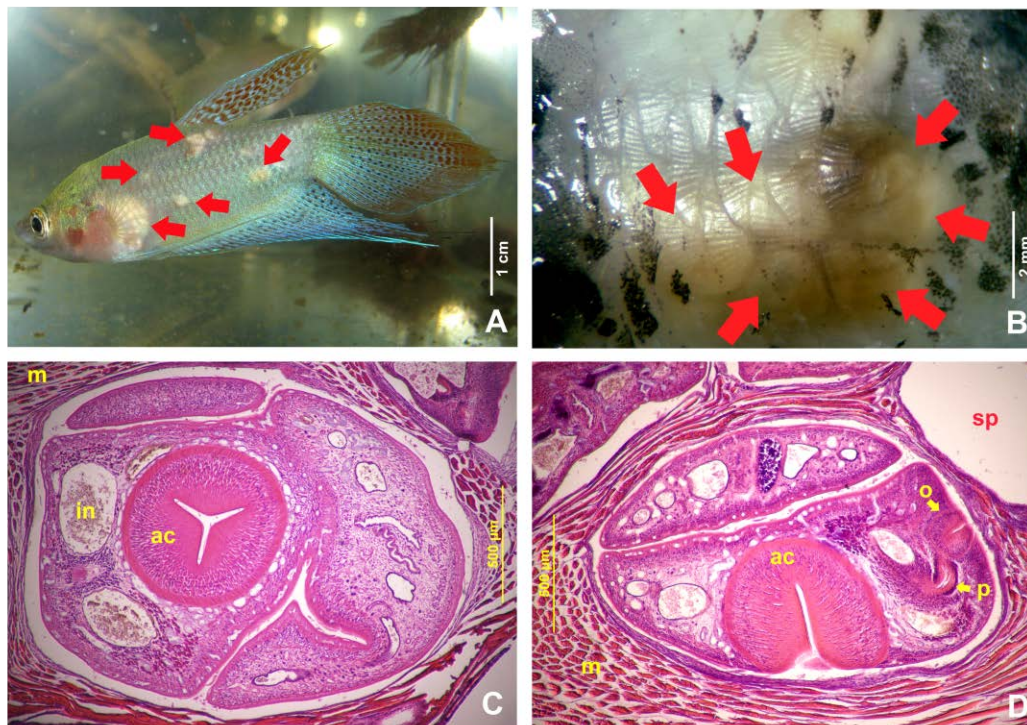
The metacercariae found in this study were identified as *Euclinostomum heterostomum* (Rudolphi, 1809) Travassos, 1928. The characteristics of *E. heterostomum* cyst were yellowish and spherical, anterior and posterior ends rounded, excretory vesicle and intestinal caeca brownish, intestinal caeca are observed on the external surface. Entire *E. heterostomum*, body length  $7.10 \pm 0.49$  (5.78 - 7.77) mm, width  $2.52 \pm 0.14$  (2.25 - 2.73) mm. Oral sucker small, rounded and subterminal, length  $187.40 \pm 9.74$  (167 - 199)  $\mu\text{m}$ , width  $248.40 \pm 11.25$  (223 - 264)  $\mu\text{m}$ . Ventral sucker well developed, larger than oral sucker length  $917.30 \pm 46.35$  (821 - 978)  $\mu\text{m}$ , width  $873.65 \pm 43.87$  (780 - 930)  $\mu\text{m}$ , located at the first third of the body. Pharynx length  $103.80 \pm 4.64$  (94 - 111)  $\mu\text{m}$ , width  $132.35 \pm 5.75$  (120 - 141)  $\mu\text{m}$ . Male reproductive system consisted of anterior testis and posterior testis, located in the second half of the body. Anterior testis, U-shaped, length  $157.25 \pm 10.54$  (135 - 179)  $\mu\text{m}$ , width  $618.20 \pm 35.90$  (530 - 680)  $\mu\text{m}$ . Posterior testis, triangle-shaped, length  $332.40 \pm 21.91$  (295 - 381)  $\mu\text{m}$ , width  $445.65 \pm 24.92$  (384 - 491)  $\mu\text{m}$ . Ovary lies between the two testes, length  $158.40 \pm 11.34$  (136 - 179)  $\mu\text{m}$ , width  $126.80 \pm 9.36$  (110 - 148)  $\mu\text{m}$ . Intestinal caeca, branched with about 10 - 12 simple lateral blind diverticula.

Histopathological section of three infected osphronemid fish species showed metacercariae invaded into the muscular tissue and was encapsulated by sheets of connective tissue within the host musculature (**Figures 1C-1D**).

Genus *Euclinostomum* Travassos, 1928 (Synonyms *Tumaclinostomum* van der Kuyp, 1953; *Metaclinostomum* Pande and Baugh, 1970) was reported to be found in the buccal cavity and

esophagus of piscivorous birds (final host) in Europe, Asia, Africa, America [8]. Seventeen species of *Euclinostomum* have been reported namely *E. africanum* (Stossich in Galli-Valerio, 1906), *E. ardeolae* El-Naffar and Khalifa, 1981, *E. bhagavantami* Jaiswal, 1957, *E. channai* Jaiswal, 1957, *E. clarias* (Dubois, 1930), *E. dollfusi* Fischthal and Kuntz, 1963, *E. gastrocaecum* Bilqeess, 1972, *E. heptacaecum* Jaiswal, 1957, *E. heterocaecum* Bilqeess, 1972, *E. heterostomum*, *E. indicum* Bhalerao, 1942, *E. lauroi* Tendeiro, Travassos Santos Dias and Fazendeiro do Carmo Martins, 1974, *E. minutus* Zaidi and Khan, 1975, *E. multicaecum* Tubangui and Masilungan, 1935, *E. nephrostomum* Bilqeess, 1972, *E. vanderkuypi* Fischthal and Kuntz, 1963. Recently, metacercaria form *Euclinostomum robustum* was found in freshwater fish in Pakistan [9]. However, three species of *Euclinostomum*, *E. bhagavantami*, *E. channai* and *E. heptacaecum* change their taxonomy to another genus *Metaclinostomum* (*M. bhagavantami*, *M. srivastavai* and *M. heptacaecum*, respectively) [10]. *E. dollfusi* and *E. vanderkuypi* are synonyms of *E. heterostomum* [11].

This is the first report of the *E. heterostomum* metacercarial infection in muscle of osphronemid fishes. *E. heterostomum* is widely dispersed throughout Europe, Asia and Africa [6]. The wide geographical distribution and high infection rates of clinostomatids might be related to the wide range of suitable first intermediate hosts (gastropods), second intermediate hosts (fishes) and final hosts (several piscivorous birds) [12-15]. *E. heterostomum*'s life-cycle had been well documented [16-17]. The snails, *Bulinus (Physopsis) globosus* (Morelet), *Bulinus truncatus* (Audouin) and *Indoplanorbis exustus* (Deshayes) are regarded as the first intermediate host [16-18], while piscivorous birds are found as the final hosts [15,17,19]. Nevertheless, some species of *Euclinostomum* are not cosmopolitan parasites such as *E. africanum* reported in Africa [20], *E. ardeolae* reported in Egypt [21], *E. indicum* reported in India [22] and *E. robustum* found in Pakistan [9].



**Figure 1** Osphronemid fishes infected with metacercariae of *Euclinostomum heterostomum*.

A shows the numerous metacercariae observed in the muscle tissues (arrows)

B is *Trichopsis vittata* after removal of scales, a group of encysted metacercariae is found. This group consisted of 6 metacercariae (arrows).

C-D are the histopathological sections of osphronemid fish muscle from *Euclinostomum heterostomum* metacercariae infestation. Metacercariae encapsulated by sheets of connective tissue in the muscle. (C from *Trichopsis vittata*, Phuket province; D from *Trichopsis vittata*, Ranong province)

ac = acetabulum, in = intestinal ceca, m = muscle, o = oral sucker, p = pharynx, sp = space after removed metacercariae.

Some metacercariae are specific in some fish such as *Cotylurus erraticus* (Rudolphi) parasites in fishes of the genus *Coregonus*, particularly *C. albula* (Linnaeus) and *C. lavaretus* (Linnaeus) [23], some species of *Euclinostomum* showed host-specificity at the genus level such as *E. clarias* metacercaria found from fish in *Clarias* species, viz., *Clarias agboyiensis*, *Clarias angolense*, *Clarias ebriensis*, *Clarias gariepinus* [24-26]. But *E. heterostomum* has a wide range of freshwater fish hosts, exemplified by *Channa punctatus* (Bloch), *Oreochromis mossambicus* (Peters), *Oreochromis niloticus* (L.), *Sarotherodon melanotheron* (Ruppell), *Tilapia zilli* (L.) [1,14,16-17,27-29]. Some species of *Euclinostomum* were located in various organs and in various host

species, for example, *E. multicaecum* was found in the liver, muscles, kidney, pharyngeal wall, gill cavity and external surface of the alimentary canal of *Anabas testudineus*, *Channa punctata* [30-33], *Channa striata* [34-35], *Heteropneustes fossilis*, *Mystus tengara*, *Nandus nandus* and *Ompok pabda* [30] in Bangladesh, India, the Philippines and Vietnam [35-37]. *E. heterostomum* metacercariae has also been reported in several organs such as the buccal cavity, skin, eyes, coelomic cavity, liver, spleen and kidney [28-29], but only muscles were found to be infected in osphronemid fishes in this investigation. In some cases, more than one species of *Euclinostomum* metacercariae were found in same fish such as *E. gastrocaecum*, *E.*

*heterocaecum*, *E. nephrostomum* and *E. indicum* from *Channa marulius* [11].

This metacercarial infection shows no mass mortality of infected fish but in many cases the infection led to a decrease in body weight and mortality [38]. The clinostomatid cysts may infect the skin, muscle and visceral organs which can induce severe damage that would impact the gross and histopathological effects in fish [18]. However, there have been reports in the case of massive metacercarial infection which have caused mass mortalities in young fish [18]. Moreover, an infection experiment of *E. heterostomum* in *O. mossambicus* fingerlings and mortality was observed under hyperinfecting conditions [16].

In Thailand, only *Euclinostomum* sp. has been reported in guppy *Poecilia reticulata* Peters in Songkhla province [39] and Chonburi province [40]. The presence of *E. heterostomum* in Thailand is thus a new host record for this parasite species.

### Conclusion

This was the first report of the *E. heterostomum* metacercarial infection in muscle of *Trichopsis vittata*, *Betta splendens* and *Betta imbellis* and also the first report of this parasite species in Thailand. However, this metacercaria did not seriously affect fish mortality, although, the parasite are found in high quantities in fish muscle.

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