

# Experimental Infection with Xiphidiocercariae and Surveying Aquatic Product infection with Metacercariae

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## Research Article

**Keywords:** Gie, Metacercaria, Trematode, Opsariichthys pachycephalus

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# 1 Experimental Infection with Xiphidiocercariae and Surveying Aquatic Product

## 2 infection with Metacercariae

### 3 Abstract

4 The artificial gastric-juice digestive method was used to examine 250 preserved  
5 clams, 125 living *Corbicula fluminea* samples, and 53 preserved fish that were all  
6 bought randomly from markets. We did not observe any metacercariae in the products  
7 bought from traditional markets. However, this does not indicate that preserved aquatic  
8 products can be sold without being cooked because, we observed that self-infected  
9 metacercariae survive for 5 minutes after being digested in fish bodies can still at a  
10 temperature of 50°C, but survive for only 1 minute when the temperature rises to 80°C.  
11 In an experimental infection study, we determined that *Corbicula fluminea* was not  
12 infected with xiphidiocercariae, but the fins of *Opsariichthys pachycephalus* can be  
13 infected and experimental metacercariae were observed. *Austropeplea ollula* was  
14 determined to be the first intermediate host of the xiphidiocercaria parasite, and  
15 *Opsariichthys pachycephalus* is a suitable second intermediate host of trematodes.  
16 Therefore, raw or insufficiently cooked, they pass down parasitic diseases.

17  
18 **Key words:** Gie, Metacercaria, Trematode, *Opsariichthys pachycephalus*

### 19 Introduction

20 Food-borne trematode infection is a global problem (Fried et al., 2004; Chai and  
21 Lee, 2002; Keiser and Utzinger, 2005; Fried and Abruzzi, 2010; Chai et al., 2012; Rim  
22 et al., 2013), and causes various clinical infections in humans. Cercaria and  
23 metacercaria play an important role in the transmitting cycle of the trematode. There are  
24 many secondary hosts in many kinds of aquatic insect larva and freshwater fish (Ono,  
25 1935; Radomyos et al., 1989; Hong et al., 1996).

26 Mollusks and crustaceans are important components of the traditional diet in  
27 Taiwan. Gie is a preserved food, treated with salts, sugars, rice wines, vinegar, soy-bean  
28 sauces, and other condiments to preserve the meat or vegetables in traditional food.  
29 Preserved clams and gie are traditional treatments with mixed sources. The pickled food  
30 had a special flavor that can promote appetite. Such as the juice of the crushed crayfish  
31 had been used as a remedy for measles in Korea (Choi, 1990), Kimchi and crab with  
32 wines in Korea and China (Loh, 1966; Choi, 1990); Salted fish paste was used to  
33 prepare raw or half-cooked seafood after treatment with salt in Philippines  
34 (Tangtrongchitr and Monzon, 1991). The food is most associated with Asian cuisine,  
35 which features an assortment of preserved seafood pasta and sauces.

36 A past report revealed that a woman had never traveled outside the continental  
37 United States but became infected while eating raw fresh-water fish (sushi) that had  
38 been served at a local Japanese restaurant (Adams et al., 1986). Since immersion of the

39 infected crustacean host in rice wine or strong brine for a short time will not kill the  
40 metacercaria. The infested crustaceans are boiled or fried in deep oil to ensure against  
41 infection (Loh, 1966; Kim, 1984; Lee and Nam, 1977).

42 In this study, we attempt to know the parasite infection of the preserved food. In  
43 the post paper, metacercaria of *Metagonimus yokogawai* was determined in the scare of  
44 the fresh-water fish, including *Opsariichthys pachycephalus*, *Zacco platypus*,  
45 *Distoechodon turmirostris*, *Varicorhinus barbatulus*, *Hemibarbus labeo*, and  
46 *Acrossocheilus formosanus* in Taiwan (Li et al., 2013). Above all fresh-water fish was  
47 cooked with fire. Higher temperatures will kill the metacercaria of the scare. The  
48 preserved fish treated with salts, sugars, rice wines, vinegar, and soy-bean sauces has  
49 not killed the metacercaria but can make the delicious foods.

50

## 51 **Materials and methods**

52 Intermediate hosts collection and examination

53 250 marinated river clams (*Crobcicula fluminea*), 125 living *Crobcicula fluminea*,

54 and 53 preserved fish (*Crossostoma lacustre* (Steindachner)) (Fig. 1) were random

55 collect from traditional markets in Miaoli County, Taiwan. 15 freshwater fish

56 (*Opsariichthys pachycephalus*) was collected from Dari River, Taichung, Taiwan. *O.*

57 *pachycephalus* and other 31 experimental fish *C. auratus* were anesthesia with ether and

58 examined the fin of the collected freshwater fish under the dissection microscopic to  
59 confirm metacercaria-free in the fin of the fishes. Artificial gastric juice was used to  
60 collect the metacercariae.

61

62 Cercaria isolation and experimental infection

63       278 *Austropeplea ollula* were random sampling from Dakeny stream, Taichung,  
64 Taiwan. The light-lamination method was used to recover cercaria from freshwater  
65 snails at 25°C for 2 hr. Recovery cercaria (Fig. 2) were used to infect the secondary  
66 host including, *C. fluminea*, *O. pachycephalus*, and *Carassius auratus* to confirm the  
67 infection recovery rate of the experimental cercaria infection, as the positive control of  
68 the activity of isolated cercaria. All experimental secondary intermediate hosts were  
69 sacrificed after 3 days postinfection.

70

71 Metacercaria isolation

72       Artificial gastric juice was used to isolate the metacercariae from the experimental  
73 secondary intermediate host. Briefly, flesh tissue is cut into small piece and then adds  
74 ten times and equal volume of artificial gastric juice is into the flask. The flask was  
75 incubated at 25, 50, and 80°C for 3 to 4 hours and keeps stirring. 300µm plastic filter  
76 was used to remove the residue. The filtered fluid was placed into a 500-ml beaker to

77 which tap water was added and standing for 20 to 30 min. The supernatant was replaced  
78 with tap water. The above process was repeated several times until the supernatant was  
79 clear and the sediment was observed under a dissection microscopic to collect the  
80 metacercariae. Isolated metacercariae were treated at 80°C to evaluate the effect of the  
81 temperature.

82

### 83 **Result**

#### 84 Samples examination

85 Non-metacercaria was observed from 250 marinated river clams, 125 live river  
86 clams, and 53 preserved fish. The pH of the source of the marinated fluid is 4.7.

87 Diatomaceous was isolated after digestion.

88

#### 89 Experimental infection

90 Non-metacercaria was observed from experimental clams that infect with  
91 xiphidiocercaria. The metacercariae were obtained in the fin of the fish and the recovery  
92 rate of the metacercaria is 73.3% (11/15) in *O. phchycephalus* (Fig. 3). The recovery  
93 rate of the metacercaria is 32.3% (10/31) after day 1 post-infection and 90.3% (28/31)  
94 after day 3 post-infection in *C. auratus*.

95

96 Temperature effect

97 No effect was observed in the metacercaria experiment at 25°C temperature  
98 incubation. Metacercariae keep activate under 50°C for 5 minutes and were surviving  
99 only one minute after being treated with 80°C incubation.

## 100 **Discussion**

101 Trematode infection is a public health problem, particularly in Southeast Asia and  
102 the Western Pacific region (Keiser and Utzinger, 2005). The meat of fish can be infected  
103 with trematodes (Dorny et al., 2009). We evaluated the trematode infection of the  
104 preserved foods, sampling from the traditional markets and used to exams. Our results  
105 showed that non-metacercaria was found in 250 marinated river clams (*C. fluminea*),  
106 125 living *C. fluminea*, and 53 preserved fish (*Crossostoma lacustre* (Steindachner).  
107 Metacercaria can break with 80°C heat in the making process of the preserved clams. In  
108 order to increase the taste of the delicious clams reserved directly with the live clams,  
109 most likely to infect other species of intestinal flukes that an infected people.

110 On the other hand, the wild clam is already nearly extinct, pickled clams are  
111 commercially farmed mostly for breeding sites. In the breeding process, there is little  
112 opportunity to come into contact with the intestinal trematode reservoir hosts, including  
113 fish-eating birds as well as dogs and cats serve as the reservoir host (Li et al., 2010,  
114 2013), thus reducing the chance of infection by intestinal parasites.

115 Preserve to the date of manufacture of minced meat inspection has about two and a  
116 half months, although the manufacturing process is not cooked, indirect pickled fish,  
117 even if there are metacercariae but may also be due to pH, and on a sauce of vinegar, the  
118 wine is more sensitive or more term survival of metacercariae died. A previous report  
119 showed that boiling the crayfish for 30 seconds or soaking in Japanese soy sauce for  
120 about 1 week killed all of the metacercariae (Loh, 1977). This indicates eating raw  
121 pickled foods, the longer its date of manufacture, the less opportunity to be infected,  
122 such as the unfortunate buy just made pickled food consumption is likely to be infected.

123 In a previous study, metacercarial infectivity was recorded in the laboratory-reared  
124 *C. fluminea* exposed to the cercariae of *Echinostoma cinetorchis* (Chung et al., 2001).  
125 Although, *C. fluminea* can be a potential secondary intermediate host of *E. cinetorchis*.  
126 In this study, *C. fluminea* will become the second intermediate host like this could not  
127 be confirmed, but *C. fluminea* will not be infected with xiphidiocercariae. However, the  
128 river clam is not a secondary host of xiphidiocercariae because of its specificity. This  
129 has been confirmed in the present study.

130 In experimental river fish (*O. pachycephalus*) and *C. auratus*, we determined that  
131 the total infection rate of the metacercariae was 45.7% (21/46) after day 3 post-infection.  
132 The metacercaria infection rate of *O. pachycephalus* was higher than *C. auratus*. We  
133 suggested that *O. pachycephalus* was a suitable intermediate host of fish-borne



134 trematoda. This result was the same as Li (2013) reported for *M. yokogawai*  
135 metacercaria infection. Additionally, infected fins were placed in petri dishes with  
136 marinade, the result showed that the fins on the metacercariae killed decomposition  
137 after two hours of incubation, similarly to gie mince. However, metacercariae were  
138 more sensitive to a sauce of vinegar and wine. The long-term preserved date can avoid  
139 infecting the marinated river clams and preserved fish. We suggested that pickled clams  
140 and fish in salted boiling water boiled (Loh, 1966; Lee and Nam, 1977) and keeps  
141 long-term preserved before diet that can make delicious food and avoid parasite  
142 infection.

143

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147

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Fig. 1 Preserved fish (*Crossostoma lacustre* (Steindachner))

208

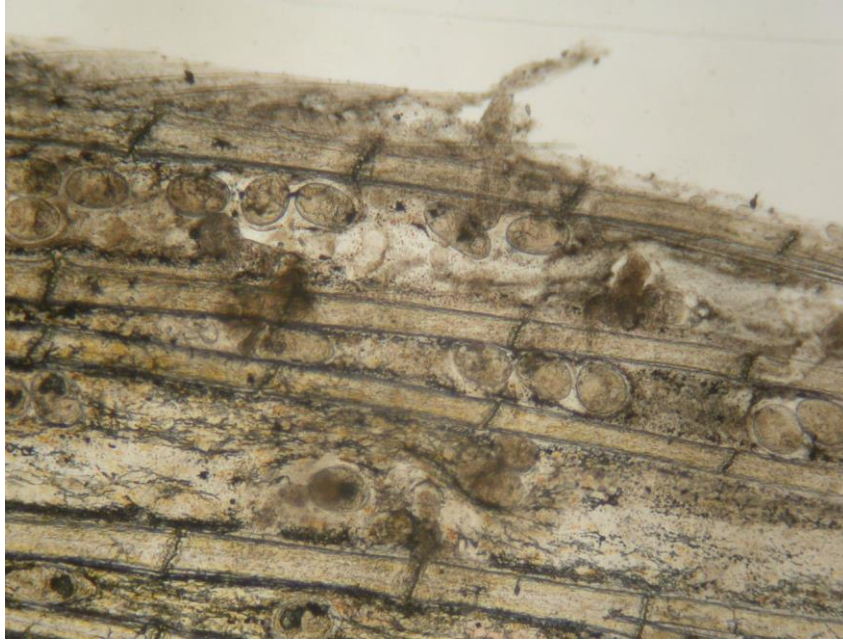


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Fig. 2 Xiphidiocercaria was collected from *Austropeplea ollula*

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212

213

Fig. 3 Metacercariae were observed in the fin of *Opsariichthys pachycephalus*