

Studies on Intestinal Trematodes in Korea

II. Identification of the Metacercariae of *Heterophyes heterophyes nocens* in Mulletts of three Southern Coastal Areas

Byong-Seol Seo, Seung-Yull Cho, Jong-Yil Chai and Sung-Tae Hong

Department of Parasitology and Institute of Endemic Diseases,
College of Medicine, Seoul National University

INTRODUCTION

As well known, the fluke family Heterophyidae inhabit the intestine of fish-eating birds and mammals and are characterized by their minute size of adult worms. The heterophyid flukes ever reported from human beings could be categorized into 4 subfamilies, i.e., Heterophyinae, Metagoniminae, Centrocestinae and Haplorchiinae, as well as into 7 genera, i.e., *Heterophyes*, *Metagonimus*, *Stellantchasmus*, *Centrocestus*, *Pygidiopsis*, *Haplorchis* and *Procerovum*, and into more than 20 species (Ito, 1964a). Medical attention has been paid to these small flukes not only because they are parasitic in human intestine but because *Heterophyes*, *Stellantchasmus* and *Haplorchis* spp. were reported to have allowed visceral spreading of the eggs and caused erratic parasitism, frequently of fatal, in Philippines and in Japan (Africa et al., 1940; Nagano et Inoue, 1955).

Heterophyes is the type genus of the family and several species were recorded from man, dog or cat in Mediterranean Coasts along Nile Delta of Egypt and in Far East (Loose, 1902; Onji et Nishio, 1916; Ozaki et Asada, 1926; Witenberg, 1929).

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The distribution of *Heterophyes* sp. in Korea had been claimed by some workers at earlier times by their short comments on finding of adult worms (Muta, 1914; Kobayashi, 1925; Faust et Nishigori, 1926; Furuyama, 1930), which were not validated through any description of the adult worms. And no other literature is available evidently to verify the existence in Korea, based on the morphological identification of adult worm. Recently Chun (1960) reported erroneously the *Heterophyopsis continua* worms under the name of *Heterophyes continus*, from experimental infection of the metacercariae which were isolated from brackish water fish, *Lateolabrax japonicus*.

In the present study, authors attempted to describe newly the metacercariae of *Heterophyes* sp. from mulletts collected in the three southern coastal areas in Korea and the adult worms which were identified as *Heterophyes heterophyes nocens* after experimental infection.

MATERIALS AND METHODS

1. Collection and observation of the metacercariae

As shown in Table 1, a total of 109 mulletts, *Mugil cephalus* of three areas, i.e., Hadan Dong, Pusan City; Geoje Island, South Kyongsang Do and Yongsanpo Eup, South Cholla Do, Korea (Fig. 1), were purchased at fish markets of

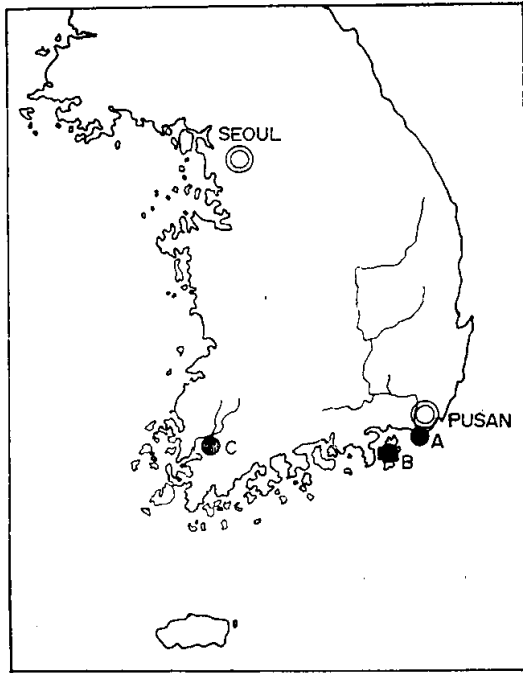


Fig. 1. Map of southern Korea showing the areas where mullets were collected.

- A: Hadan Dong, Pusan City,
- B: Geoje Island, South Kyongsang Do,
- C: Yongsanpo Eup, South Cholla Do.

respective areas during the period from May 1978 to October 1979 and examined for heterophyid metacercariae either by muscle compression method or by digestion technique.

As there were two or more kinds of heterophyid metacercariae in muscle of the mullets, those of *Heterophyes* sp. only were collected from the digested muscle fragments counted under dissecting microscope and subjected in this study.

Several metacercariae were excysted by compressing them with cover slip and fixed with 10% formalin for measurement and photograph. The excysted ones were stained with Semichon's acetocarmine and the developmental status of the genital organs was observed.

2. Experimental infection of the metacercariae and obtaining adult worms

Three mice and four rats were infected with

a total of 580 metacercariae of *Heterophyes* sp. to obtain adult worms (Table 2). Experimental infection was carried out by feeding the metacercariae, through polyethylene capillary tube, into stomach of the rodents under ether anesthesia. After 3 to 20 days from infection, the rodents were killed and the whole intestine was searched for worms in 37°C saline solution.

Some of the living adult worms were observed under cover slip pressure and fixed with 10% formalin. Some fixed specimens were measured and photographed immediately, and stained with acetocarmine.

As the eggs were not found by stool examination of the experimental animals, they were obtained for measurement from the distal portion of uteri of some worms.

RESULTS AND WORM DESCRIPTION

1. On the metacercariae identified as *H. heterophyes nocens*

The infection rates of the mullets with the metacercariae of *H. heterophyes nocens* were relatively low and ranged from 5.9% to 12.7% according to areas (Table 1). A total of 821 metacercariae were collected from 11 infected mullets with wide individual variation in metacercarial burden, 1 to 480 per fish.

Table 1. Infection rate of *Mugil cephalus* with the metacercariae of *H. heterophyes nocens*

Mullets from	No. exam.	No. posit. (%)	Total No meta-cercaria (Mean)
Hadan Dong	55	7(12.7)	322 (46)
Geoje Island	20	2(10.0)	4 (2)
Yongsanpo	34	2 (5.9)	495(248)
Total	109	11(10.1)	821 (75)

The metacercariae were found most frequently in muscles beneath the pectoral fin of the mullets than any other part. Infected mullets

were found during May to August in two years, although the collection of mullets was performed nearly every month during the study period.

Description of the metacercariae

H. heterophyes nocens

Cyst round (Fig. 2 and 3) or slightly ellipsoid measuring 0.155~0.198 by 0.131~0.190mm. Metacercarial wall 3.0~4.4 μ m in thickness and surrounded by muscular tissue of the mullets in compressed preparations. Excretory bladder bluish black or dark pinkish in color and filled with many granular excretory corpuscles. Three suckers prominent and muscular, one at the anterior end and the other two at the middle portion of the body (Fig. 3).

Excysted one oval in shape (Fig. 4) measuring 0.237~0.363mm long and 0.134~0.198mm wide. Tegumentary spines extended from anterior to the posterior one-third level of the body. Oral sucker subterminal and relatively large, 0.035~0.057mm in diameter. Prepharynx present and pharynx well developed measuring 0.021~0.036mm in diameter. Dark brownish pigments present making triangular areas on bilateral sides of esophagus. Esophagus and intestine not easily seen in fixed preparation. Ventral sucker not larger than the oral sucker, measuring 0.041~0.055 by 0.036~0.051mm, well developed and

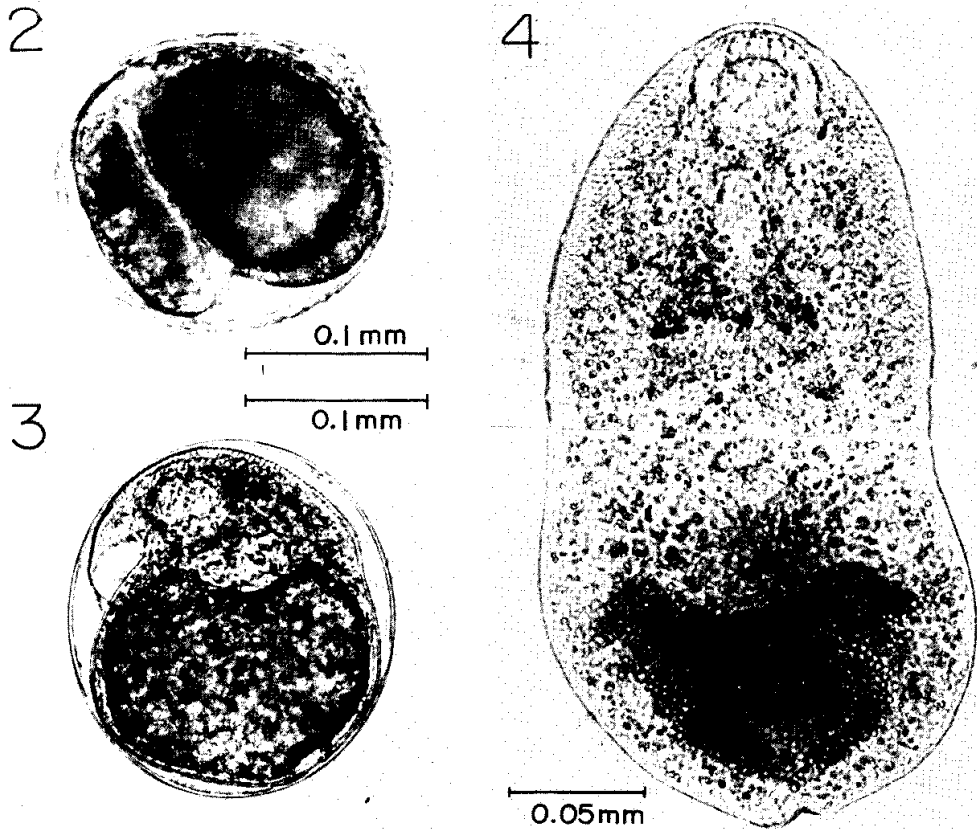
muscular. Genital sucker prominent and located diagonally on the right postero-lateral side of the ventral sucker in a dorsal view (Fig. 4), and smaller than the ventral sucker, 0.028~0.035mm in length and 0.016~0.032mm in width. Rodlets hardly discriminative or countable. Seminal vesicle invisible. Testes primordia seen in stained preparations bilaterally to the middle level of excretory bladder and 0.012~0.019mm in diameter. Ovary also developed a little and visible by staining, deviated slightly to the opposite side of the genital sucker and in front of excretory bladder. Excretory bladder very large and V-form in appearance, 0.090~0.132mm in width and 0.055~0.073mm in length, and filled with numerous corpuscles.

2. Results of experimental infection and identification of the worms

The worm recovery rates from the experimental rodents were as shown in Table 2. Eighty-eight worms were recovered from small intestine of 6 rodents among 7 infected. The mean recovery rate in 7 rodents was 15.2%. Worms older than 10 days were more frequently found in ileum than in jejunum in both kinds of host. No difference in the recovery rate was demon-

Table 2. Results of the recovery of *H. heterophyes nocens* adults from experimentally infected mice and rats

Host	No. metacercariae infected	No. recovered worms from		Total No. recovered worms(%)	Duration of infection (days)
		Jejunum	Ileum		
Mouse A	40	7	1	8(20.0)	7
Mouse B	80	1	8	9(11.3)	10
Mouse C	80	0	14	14(17.5)	13
Subtotal	200	8	23	31(15.5)	—
Rat A	65	10	0	10(15.4)	3
Rat B	75	0	0	0 (0.0)	3
Rat C	40	3	3	6(15.0)	9
Rat D	200	6	35	41(20.5)	20
Subtotal	380	19	38	57(15.0)	—
Total	580	27	61	88(15.2)	—



Figs. 2-4. Metacercariae of *H. heterophyes nocens* from mullets.

2. Unpressed one from digested muscle of mullet. 3. Slightly pressed one with a cover slip showing three suckers. The smallest one is genital sucker. Note also the black pigments near pharynx and the large excretory bladder. 4. Liberated one in dorsal view. Genital sucker is on the left lower portion of the ventral sucker in this figure.

Table 3. Size development of *H. heterophyes nocens* worms in experimental hosts according to duration of infection

Duration of infection	Host	*Body size(mm.)		No. of measured worms
		Length (mean)	Width (mean)	
3 days	Rat	0.310-0.341 (0.329)	0.142-0.234 (0.215)	3
7 days	Mouse	0.538-0.731 (0.645)	0.290-0.376 (0.319)	8
10 days	Mouse	0.559-0.699 (0.628)	0.258-0.404 (0.346)	9
13 days	Mouse	0.612-0.729 (0.671)	0.288-0.377 (0.330)	3
20 days	Rat	0.662-0.791 (0.723)	0.306-0.386 (0.345)	12

* Measurements were done exactly after fixation with 10% formalin under slight pressure with cover slip.

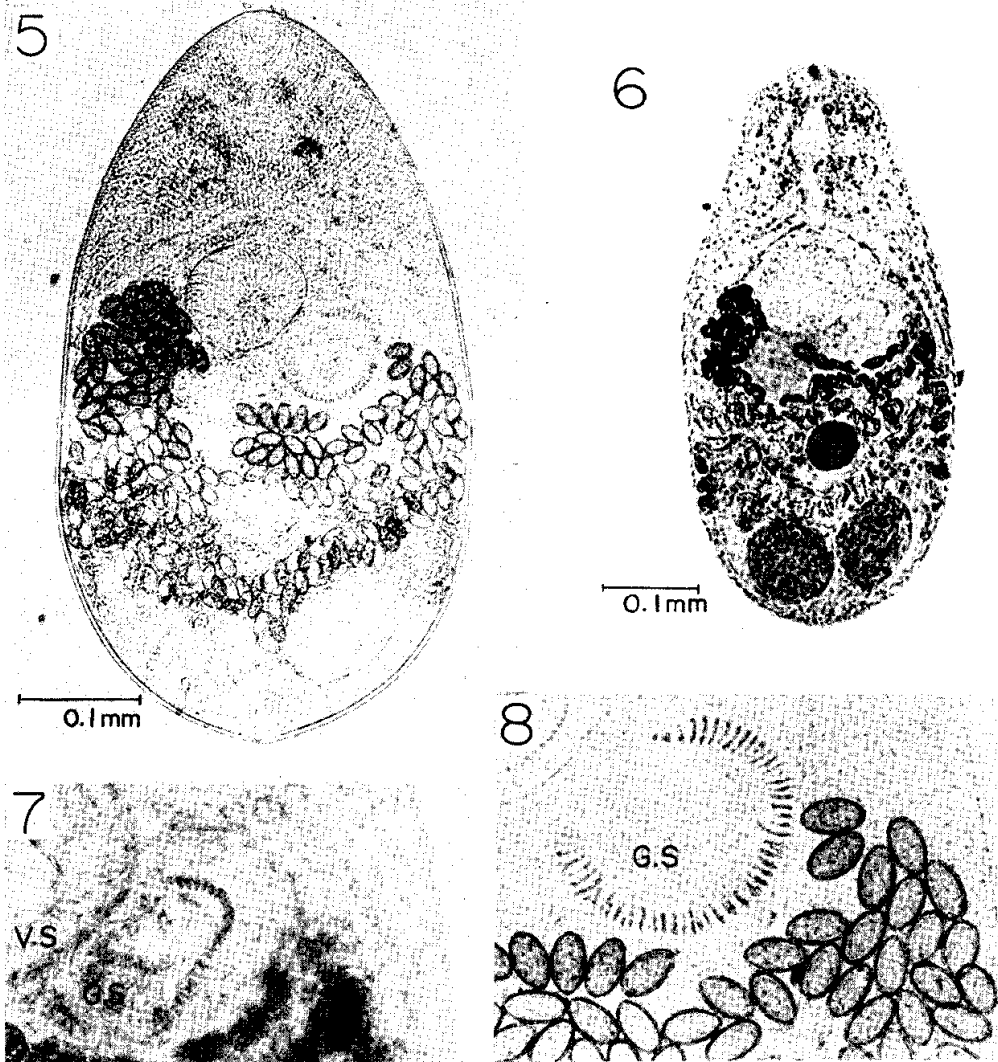
strable between mice and rats. Size of the worms increased according to duration of infection up to 20 days, as shown in Table 3.

Description of adults worms

Heterophyes heterophyes nocens(Onji et Nishio, 1916)

Measurements of 12 worms aged 20 days were described here. Body dorsoventrally flattened and ovoid in shape, 0.66~0.79mm in length and 0.31~0.39mm in width, and covered with fine scale-like spines from the anterior end to the posterior one-third level of the body. The posterior end portion appeared a little protuberant in some worms but without any more serration (Fig. 5 and 6).

Oral sucker subterminal measuring 0.060~0.080



Figs. 5-8. Adult worms of *H. heterophyes nocens* from experimental rodents.

5. 10 day worm from a mouse, unstained in ventral view. 6. Stained one, 9 day worm from a rat. 7. Magnification of the worm ($\times 500$) in Fig. 5, taken before fixation. Note constricted rodlets with contraction of genital sucker, (G.S) in which state counting of rodlets is difficult. V.S.: Ventral sucker 8. *Ibid*, taken exactly after complete fixation in dilated state of the genital sucker (G.S). In this specimen, the number of rodlets was 56~57. Eggs are seen in typical shape in the uterus.

mm in diameter. Prepharynx present and pharynx well developed, muscular and 0.040~0.043mm in diameter. Esophagus slender and muscular, and intestine bifurcating in front of the ventral sucker and ceca terminating at the mid- or post-testicular level. Two ceca more likely to terminate unequally. Excretory bladder not easily seen but excretory pore visible subterminally.

Ventral sucker well developed with its location on

median line of the body and larger than any other sucker, 0.100~0.126 by 0.086~0.123 mm in size. Genital sucker prominent, 0.080~0.116 by 0.073~0.110mm in size, with 52~62 rodlets, which are interrupted ventrally at antero-median portion and arranged along inner line of the sucker when constricted (Fig. 7) or along outer margin when dilated (Fig. 8). Genital pore visible at the interrupted portion of rodlets,

Testes ovoid or globular in shape and both nearly equal in size, 0.100~0.133 by 0.073~0.113 mm, locating side by side not exactly parallel, near the posterior end. Seminal vesicle well developed and C-shaped between the ventral sucker and the ovary. Vas deference and ejaculatory duct connected to genital pore. Cirrus pouch absent.

Ovary globular or round, located on median line of the body and in front of testes, measuring 0.047~0.073mm in diameter. Seminal receptacle not well developed and behind ovary. Laurer's canal present and vitellaria consisted of 7~10 or more follicles on each side at lateral fields between the level of seminal vesicle and testes. Uterus coiled long and located ventrally between acetabulum and testes with more than 200 eggs.

Eggs from distal uteri dark yellowish in color containing miracidia and operculated, and 0.024~0.027mm in length and 0.013~0.015mm in width. Shape of the eggs oval, similar with those of *Metagonimus yokogawai* but slender in appearance and the maximum width always at the equatorial portion.

DISCUSSION

There was some difficulty in the species identification of the worms obtained in the present study. There have been many debates on the Japanese species of *Heterophyes*, i.e., *H. nocens* Onji et Nishio, 1916, to which the presently collected worms agreed well. Cort et Yokogawa(1921) stressed the validity of *H. nocens* as a distinct one from Egyptian *H. heterophyes*, because of the smaller size of the worms, smaller number of rodlets on gonotyl, unequal size or termination of each cecum in a worm, etc. And Witenberg (1929) retained the validity of the species, although he concluded that only the number of rodlets is the distinguishable point between the two species, which is 50~60 in *H. nocens* and 75~87 in *H. heterophyes*. However, Lane(1922) and Faust et Nishigori(1926) denied *H. nocens*

and regarded it as a synonym of Egyptian species, *H. heterophyes*. On this account, Asada (1934) proposed it as a subspecies and called as *H. heterophyes nocens*, accepting the smaller number of rodlets is a valid character for such nomenclature.

Nevertheless, from then until recently, the name is not settled down even among the Japanese workers and it was called either as *H. nocens* (Ito, 1964 a; Komiya, 1965; Komiya et Suzuki, 1966) or as *H. heterophyes nocens*(Ito, 1964 a; Morishita, 1964) or even as *H. heterophyes* var. *nocens*(Ito, 1964 a; Yokogawa et al., 1965). In the authors' opinion, Japanese *Heterophyes* is valid enough to be a distinct one. In this connection, Chen(1936) stated that, in the speciation of *Haplorchis*, the arrangement and the number of chitinous spines or hooks on ventrogenital sucker was the only constant and practical difference between the species; *H. pumilio*, *H. taichui* and *H. yokogawai*. The scientific name should be simplified so far as any other differential point when the whole life cycle are not clarified. Anyway, in this study, the present authors regarded the collected worms as *H. heterophyes nocens* admitting Asada's (1934) opinion.

The measured values of the present specimens, except for the number of rodlets on genital sucker, agreed well to the descriptions both of *H. heterophyes*(Witenberg, 1929) and of *H. nocens* (Onji et Nishio, 1924). But the worms were smaller in size than either kinds described by Cort et Yokogawa(1921) and Asada(1934). The size of worms may vary according to age and host, and rodents may not be the suitable host to get the maximum growth of the worms, as is also indicated in case of *Metagonimus yokogawai* (Yokogawa et Sano, 1968; Chai, 1979).

It is proved firstly in this paper that Korean

mulletts also serve as an intermediate host of *Heterophyes* sp. as well as Egyptian (Kahlil, 1923) and Japanese mulletts (Onji et Nishio, 1916). Asada (1934) stated in his article that Kobayashi had found heterophyid metacercariae from the mulletts collected at Mokpo, South Cholla Do, Korea in 1925. However, the literature which account for such statement can not be traced at present.

The most important suggestion with this paper must be the probable prevalence of this fluke among the inhabitants of the three southern coastal areas, Korea, because they preferably eat raw mulletts than processed ones. It is expected the prevalence may not be high enough to be detected easily. Because the eggs of *Heterophyes* sp. and *M. yokogawai* are very similar in shape and the latter is the more widely distributed and well known heterophyid trematode in Korea (Seo, 1979), the eggs of the former, even if frequently appeared in human stools, may be misjudged as those of, or some variant of *M. yokogawai* without further investigation of the adult worms. However, earlier comments (Stryker, 1914; Imanishi, 1914) on the detection of *Heterophyes* sp. eggs from human stools in Korea, are not verified now because the adult worm was never identified.

Accordingly, in order to detect human cases with *Heterophyes* worms and to understand the epidemiological status of this fluke infection in the subjected areas of Korea, a keen observation should be made on the heterophyid eggs from human stool, and investigation on the adult worms should be performed.

SUMMARY

The existence of life cycle of *Heterophyes heterophyes nocens*, in three southern coastal areas of Korea, i.e., Hadan Dong, Pusan City; Geoje Island, South Kyongsang Do and Yong-

sanpo Eup, South Cholla Do, was proved by identifying the metacercariae in mulletts, *Mugil cephalus*, and by obtaining adult worms from experimental animals during the period from May 1978 to October 1979.

Infection rates of the mulletts with the metacercariae were from 5.9% to 12.7% according to areas and a total of 821 metacercariae were collected from 11 infected mulletts. And a total of 88 adult worms were recovered from small intestine of the experimental animals on the 3rd to 20th day after infection. The size of the worms increased according to the age of infection.

The adult worms were distinct with a genital sucker near the ventral sucker. A total of 52~62 rodlets are arranged in a row on the genital sucker. So they were identified as *Heterophyes heterophyes nocens* (Onji et Nishio, 1916). The possibility of prevalence of human infection in southern coasts, Korea was suggested and discussed.

—國文抄錄—

韓國의 腸吸蟲類에 關한 研究

Ⅰ. 南海岸 三個地域 崇魚에 있어서 異形吸蟲 被囊幼蟲 感染의 確證

서울대학교 醫科大學 寄生蟲學教室 및 風土病研究所
徐丙高·趙昇烈·蔡鍾一·洪性台

우리나라에 異形吸蟲屬(*Heterophyes* sp.)의 吸蟲이 존재한다는 것을 처음으로 증명하였다. 1978年 5月 부터 1979年 10月까지 釜山(하단동), 慶南(巨濟島) 및 全南(榮山浦)地方에서 구입한 崇魚(*Mugil cephalus*) 109마리중 11마리에서 異形吸蟲屬의 피낭유충이 감염되어 있음을 확인하고, 種同定을 위해 실험동물에 감염시킨 후 3日~20日 사이에 도살하여 모두 88마리의 成蟲을 얻었다.

崇魚의 皮낭유충 감염율은 지역별로 별 차이가 없었으며(5.9%~12.7%) 비교적 낮은 편이었다. 모두 580개의 皮낭유충을 감염시켰던 實驗動物(마우스 3마리 및 흰쥐 4마리)로부터의 蟲體回收率は 평균 12.5%이었고

成蟲의 크기는 감염시간이 경과할수록 증가하였으며 20日된 蟲體는 길이 0.66~0.79mm이었다. 回收한 成蟲은 生殖盤(genital sucker)이 腹吸盤의 左下方에 뚜렷이 존재하였고 52~62個의 桿狀棘(rodlet)이 生殖盤 위에 배열되어 있어서 이형흡충(*Heterophyes heterophyes nocens*)으로 同定하였다.

승어의 산지 주민들은 승어를 회로 먹는 경우가 더 많으므로 人體感染이 유행하고 있을 가능성이 크며 人體 감염예를 찾아내기 위해서는 異形吸蟲卵이 발견되면 자세히 관찰하고 치료후 성충을 얻어 감별해야 할 것으로 생각되었다.

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