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# Occurrence of *Clinostomum complanatum* in *Aphanius dispar* (Actinoptrygii: Cyprinodontidae) collected from Mehran River, Hormuzgan Province, South of Iran

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#### ABSTRACT

Objective: To investigate the possibility of Aphanius dispar (A.dispar) acting as intermediate host for Clinostomum complanatum (C. complanatum), from Mehran River, Hormuzgan Province of Iran. Methods: During a biological study of A. dispar in Mehran River, Hormuzgan Province, South of Iran, a total of 97 fish specimens were collected in 24 January 2006. Results: 4 specimens (4.12%) including 1 male and 3 female were found infected with C. complanatum metacercaria. These metacercaria were coiled in the epiderm on the body surface of infected fishes. The infection is known as yellow spot disease. The parasite abundance, intensity and prevalence were 0.05%, 1.25% and 4.12%, respectively. The infection was higher in females than males. Conclusions: To the best of our knowledge, this is the first report on occurrence of C. complanatum metacercaria in A. dispar in Iran.

### 1. Introduction

Aphanius dispar (Holly, 1929) (A. dispar) (Mahi gour-e khari, mahi dom parchami, kopurdandandar-e balehbolband in Persian) is an euryhaline tooth-carp fish[1,2] which apparently prefers brackish waters of costal areas of Iran and is also distributed in Cyprus, Iraq, Somalia, Israel, Saudi Arabia, Syria and Djibouti[3]. A. dispar occurs in shallow water and among vegetation over sand, rock or soft detritus bottoms[4,5]. Males and females in aquaria swam in separate schools[6]. This fish was responsible for the absence of mosquito larvae from streams in Karachi which otherwise appeared to be good breeding places[7,8]. Members of this family have been recorded from the area where mean temperature is about 10 °C[9]. The majority of digenetic trematodes do not pose a serious threat to fish health. However, the presence of Clinostomum spp. cysts

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(external bumps) makes the fish unattractive and can be seen by humans but normally they do not consume them[10]. The final hosts of this parasite are piscivorous birds, generally ducks or herons, in which it invades the oral cavity or pharynx. However intermediate hosts of the parasite are many fresh water fishes and snails such as *Heliosoma*, *Lymnaea* spp. in which the organism invades submucosal tissue or muscle[11,12]. The parasite cysts are surrounded by a thick capsule from the fish tegument. The infection of fish by *Clinostomum* can result in behavioral changes, disease and death, on one hand and economic loss on the other[10,13].

Since, no report was available on the occurrence of *Clinostomum* from *A. dispar* fish in Iran; the aim of this study was to investigate the possibility of *A. dispar* acting as intermediate host for *Clinostomum complanatum* (*C. complanatum*), from Mehran River, Hormuzgan Province of Iran.

#### 2. Materials and methods

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This study was conducted in Mehran river (27 ° 28′24.1″N, 57 ° 15′18.88″ E 211 Altitude) in Makran basin, near Rudan town, south of Iran, Hormuzgan Province. The bottom of this river is generally muddy with rubble, gravel and sand. Other fauna of this river are Nemacheilus, Garra and Cyprinion and the flora of Ziziphus and Tamarix are also found in the region. The water is clear and running slowly in summer but floody in winter. In this study, on 24 January 2006, a total of 97 A. dispar were collected using dip net from this river, and preserved in 10% formalin solution until examination. Total length, standard length and weight of the collected specimens were measured to the nearest 0.05 millimitre, weight of specimens to the nearest 0.001 gram and their sex was determined. Whole body of fishes was examined macroscopically for the presence of any encysted metacercaria. In case of infectivity, the locations and number of cysts were recorded. Then, the metacercariae were recovered from the cysts and fixed between two slide glasses with 70% ethanol alcohol and stained by Azocarmine. After washing in acid alcohol, dehydrated in alcohol series, cleared in xylol and mounted in Canada balsam. Body size, oral sucker and acetabulum length and width were measured. Then according to morphological characteristics, the parasite was identified.

Prevalence (%) was calculated according to the percent of infected fish divided by the total number of fish. Mean intensity was determined by dividing the total number of recovered parasites by the number of infected fish samples, while abundance was calculated by dividing the total number of recovered parasites by the number of (infected and uninfected) fish samples.

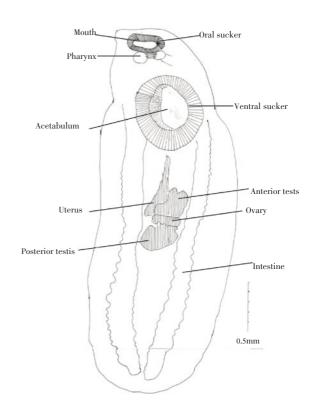
## 3. Results

A total of 97 A. dispar fish specimens (22 males and 75 females) were collected on 24 January 2006, of which 4 specimens (4.12%) including 1 male and 3 females were found infected with metacercariae of C. complanatum (Figure 1–3). Sex ratios of fishes were 3:1 in favor of females. Total and standard length of fishes ranged from 27.5–40.1 mm and 23.2–32.2 mm, respectively; and their weight ranged from 0.269 to 1.091 mg. Minimum and maximum of parasite length was to 2.92–5.81 mm; parasite width was 1.34–1.99 mm. Oral sucker length and width were 0.10–0.18 mm and 0.28–0.49 mm, respectively. Acetabulum length was 0.25–0.92 mm, and acetabulum width was 0.36–0.77 mm (Table 1). The appearance of infected fishes was disturbed with 1 to 2 cysts as external bumps on their bodies, namely yellow

grubs (Figure 1). Among 4 infected fishes, one of them was infected with two parasites and three others had only one parasite. The cysts were rounded, each containing only one metacercaria, located in the base of anal fin of fishes or on their body. Maximum and minimum length of excysted metacercariae was 5.81 and 2.92 mm, respectively (Table 1). Mean parasite length was 4.21 mm.



**Figure 1.** Photograph of *A. dispar* collected from Mehran River, Hormuzgan Province, South of Iran, infected fish with encysted *C. complanatum* metacercariae (yellow grub).



**Figure 2.** Camera lucida drawings of metacercaria of *C. complanatum* from the epiderm of *A. dispar* in Mehran River, Hormuzgan Province, south of Iran.

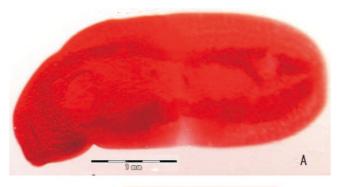
The prevalence, intensity and abundance of infection by metacercaria of *C. complanatum* were 4.12%, 1.25% and

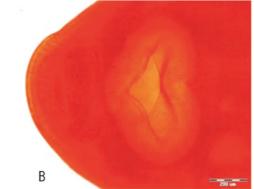
Table 1
Details of infected A. dispar with C. complanatum metacercariae in Mehran River, Hormuzgan Province, south of Iran.

Fish Lab. No.	Fish (sex)	Fish T.L (mm)	Fish S.L (mm)	Fish W	Parasite T.L (mm)	Parasite W (g)	Oral sucker L (mm)	Oral sucker W (mm)	Acetabulum length (mm)	Acetabulum width (mm)	Parasite location	Total parasite number (5)
9958	Female	33.6	27.5	0.575	4.92	1.98	0.12	0.35	0.31	0.37	base of anal fin	1
Y(5) 23	Female	27.5	23.2	0.269	3.63	1.41	0.13	0.39	0.42	0.36	base of anal fin	2
					5.81	1.99	0.10	0.28	0.92	0.77	base of pectoral fin	
9932	Female	40.1	32.2	1.091	3.74	1.55	0.12	0.38	0.44	0.51	between pectoral and pelvic fin	1
9933	Male	34.9	28.7	0.682	3.19	1.34	0.18	0.49	0.25	0.54	under pectoral fin	1

T.L= total length; S.L= standard length; W= weight and width.

0.05%, respectively. Prevalence, intensity and abundance of parasite in female and male were 0.03% and 0.01%, 1.33% and 1%, 0.041% and 0.01%, respectively. Mean parasite length and width were 4.21 and 1.65 mm, respectively.





**Figure 3.** Microscopic photos of *C. complanatum* metacercaria. A: whole body; B: oral sucker and acetabulum with higher magnification.

#### 4. Discussion

Metacercaria of *Clinostomum* have been found in many fish species, demonstrating the diversity of hosts this parasite can exploit. These fish species included *Perca fluviatilis*, *Rutilus rutilus*[9], *Salmo trutta gairdneri*[14], *Carassisus carassisus, Cyprinus carpio, Pseudorasbora parva, Cobitis anguillicaudatus*[11,15] *Carassius auratus*[16] and *Lateolabrax japonicus*[17]. Metacercaria of *Clinostomum* in addition to many fishes have also been found in toads, frogs, salamanders and tritons[18–20]. In Iran, this parasite has already been reported from *Aphanius sophiae*[21] and *Capoeta capoeta gracilis*[22]. However, in this study, first report on occurrence of *C. complanatum* metacercaria in *A. dispar* in the country is recorded.

According to the results of the present study, the highest number of metacercaria counted in *A. dispar* was 2 cysts, each one containing only one parasite; while Hosseini<sup>[21]</sup> reported as much as 14 cysts in *A. sophiae*.

In the current study, both the prevalence of infection and the intensity of infection with *C. complanatum* were higher in females than males of *A. dispar*. The prevalence was 0.03% *vs* 0.01% and the intensity of infection was 1.33% *vs* 1% in female and males, respectively. Hosseini<sup>[21]</sup> reported prevalence of

infection in females and males of *A. sophiae* to be 53.5% and 32.9%, respectively. Clinostomum prevalence rate was 50.2% in *Tilapia nilotica* in Beni–Suef[23] and 16.26% in *Poecilia reticulate*[24]. Kalantan *et al*[25] also reported the higher infection of females of *A. dispar* with the metacercaria of *C. complanatum* than males (55.9% vs 47.5%). In another study the higher infection of females of *A. dispar* with *Ligula intestinalis* plerocercoids has also been stated[26]. More research is required to elucidate the reason for higher affinity of these parasites to females.

In the current study, C. complanatum cysts in A. dispar were found in the base of anal, pectoral fins and between pectoral and pelvic fins. Hosseini[21] reported such cysts distributed in the anterior of anal and ventral fins and gills in A. sophiae. In Rhamdia guatemalensis, metacercaria of C. complanatum were found in the gills, operculum and muscle[27]. Vianna[13] reported that metacercaria in Rhamdia cf. quelen distributed in the head region and in the base of the fins, mainly those of pectoral and caudal. Kalantan et al[21] detected cysts in A. dispar mostly in the trunk region, i.e. between the origin of pectoral fin and anus (47.3%) and secondly in that of the head. These authors mention this issue can be explained on the basis of the enterance of cercariae with water current through the opercula, so it may be easier for the cercariae to penetrate the soft tissue lying under the operculum than on the scaly surface.

If a human accidentally consumes raw or semi-cooked infected fish, the fluke attaches to the mucus memberane of the human oesophagus and causes injury<sup>[12]</sup>. This parasite can cause laryngopharyngitis and even death by asphyxia in humans. Kamo *et al*<sup>[28]</sup> reported the occurrence of anemia in a Thailandese male whose eyes were infected by *Clinostomum* sp.. There are also 15 cases reported from Japan and Korea that the parasites firmly attaches to the mucus memberane of the pharynx in humans<sup>[16,29–31]</sup>. The primary therapy is removal of the parasitic worm, however, it is often difficult because of its rapid movement and firm adhesion to the submucosa<sup>[32]</sup>.

Most people are not aware of infection danger and may consume this fish in raw. So, it is necessary to inform people, and also environment organization to conserve this important species.

Further studies in the region on other fish species and their potential hazard for human infectivity is very important for a successful infection control strategies.

#### **Conflict of interest statement**

We declare that we have no conflict of interest.

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#### References

- [1] Lotan R. Osmotic adjustment in the euryhaline teleost *Aphanius dispar* (Cyprinodontidae). *Z Vgl Physiol* 1971; **75**(4): 383–387.
- [2] Andrews DA. *Aphanius dipar* (sic) a brackish water killie. *J Am Killifish Asso KN Feature* 1983; **16**(1): 24–26.
- [3] Etemadfar AR, Motabar M, Wossughi Gh. A biological and ecological survey on the geographical distribution of *Aphanius dispar* as larvivorous fish at the southern parts of Iran. *J Vet Fac Univ Tehran* 1983; **38**(2–4): 1–12. (In Persian).
- [4] Coad BW. Freshwater fishes of Iran. Acta Sci Nat Acad Sci Brno 1995; 29(1): 1-64.
- [5] Coad BW. Systematics of the tooth-carp genus Aphanius Nardo, 1827 (Actinopterygii: Cyprinodontidae) in Fars province, southern Iran. Biol Bratis 1996; 51(2): 163-172.
- [6] Al-Daham NK, Huq MF, Sharma KP. Notes on the ecology of the genus Aphanius and Gambusia affinis in southern Iraq. Freshwater Biol 1977; 7(3): 245–251.
- [7] Steven WSR. Report on an investigation in regard to the prevalence of malaria amongst the troops stationed at Karachi. J R Army Med Corps 1913; 24: 251–261.
- [8] Abd El-Galil MAA, Kamel MA, Abd El-Rahman HH, Fouda, M. A trial for treatment of yellow grub disease in *Oreochromis niloticus* by praziquantel. *Egypt J Aquat Biol Fish* 2007; 11: 801–815.
- [9] Grabda-Kazubska B. Clinostomum complanatum (Rudulphi, 1819) and Euclinostomum heterostomum (Rudolphi, 1819) (Trematoda: Clinostomidae), their occurrence and possibility of acclimatization in artificially heated lakes in Poland. Acta Parasitol Pol 1974; 22(24): 285-293.
- [10] Silva-Souza AT, Ludwig G. Parasitism of Cichlasoma paranaense Kullander, 1983 and Gymnotus carapo Linnaeus, 1814 by Clinostomum complanatum (Rudolphi, 1814) metacercariae in the Taquari river. Braz J Biol 2005; 65: 513-519.
- [11] Aohagi Y, Shibahara T, Kagota K. Clinostomum complanatum (Trematoda) infection in freshwater fish from fish dealers in Tottori. Jpn J Vet Med Sci 1993; 55(1): 153-154.
- [12] Szalai AJ, Dick TA. Helminths of stocked rainbow trout (Salmo gairdneri) with reference to Clinostomum complanatum. J Wildl Dis 1988; 24(3): 456–460.
- [13] Vianna RT. Clinostomum complanatum (Rudolphi, 1814) em Rhamdia cf. quelen (Quoy and Gaimard, 1824), no Arroio Sarandi, Rio Grande, RS. M.S. Thesis. Universidade Federal de Santa Maria, Santa Maria, RS; 2001.
- [14] Yamaguty S. *Systema helmintum*. New York: Inter Science Publication Inc; 1933.
- [15] Aohagi Y, Shibahara T, Machida N, Yamaga Y, Kagota K. Clinostomum complanatum (Trematoda: Clinostomatidae) in five new fish hosts in Japan. J Wildl Dis 1992; 28(3): 467–469.

- [16] Chung DI, Kong HH, Moon CH. Demonstration of the second intermediate hosts of *Clinostomum complanatum* Korea. *Korean J Parasitol* 1995; 33(4): 305–312.
- [17] Aohagi Y, Shibahara T, Kagota K. Metacercariae of Clinostomum complanatum found from new fish hosts, Lateolabrax japonicus and Leuciscus hakonensis. Jpn J Parasitol 1995; 44(4): 340–342.
- [18] Yamaguti S. Synopsis of digenetic trematodes of vertebrates. Tokyo: Keigakur Publishing Company; 1971, p. 1072.
- [19] Muzzall PM. Helminth infracommunities of the Newt, Notophthalmus viridescens, from Turkey Marsh, Michigan. J Parasitol 1991; 77(1): 87-91.
- [20] Goldberg SR, Bursey CR, Cheam H. Helminths of two native frog species (*Rana chiricahuensis*, *Rana yavapaiensis*) and one introduced frog species (*Rana catesbeiana*) (*Ranidae*) from arizoana. *J Parasitol* 1998; 84(1): 175–177.
- [21] Hosseini SH. The study of infection of fish with *Clinostomum* sp. in the lake of south of Tehran. PhD Dissertation University of Tehran (In Persian); 1987.
- [22] Malek M, Mobedi I. Occurrence of Clinostomum complanatum (Digenea: Clinostomidae) in Capoeta gracilis (osteichthys: Cyprinidae) from Shiroud River, Iran. Iran J Public Health 2001; 30(3-4): 95-98.
- [23] Abd El-Galil MAA, Kamel HH, Abd El Rahman AH. Effect of yellow grub disease on fish health. Sci Cong Fac Vet Med Asiut Univ Egypt 2006; 12: 8-10.
- [24] Prasadan PK, Devi ARS. Yellow grub disease in the ornamental fish, Poecilia reticulata (Poeciliidae). J Exp Zool 2007; 10(2): 405-407.
- [25] Kalantan AMN, Arfin M, Nizami WA. Seasonal incidence and pathogenicity of the metacercariae of *Clinostomum complanatum* in *Aphanius dispar. Jpn J Parasitol* 1987; 36(1): 17-23.
- [26] Gholami Z, Akhlaghi M, Esmaeili HR, Payghan R. Infection of Aphanius dispar (Holly, 1929) with Ligula intestinalis plerocercoids in Mehran River, Hormuzgan Province, south of Iran. Iran J Fish 2010.
- [27] Perez-Ponce De Leon, Osorio-Sarabia D, Garcia-Prieto L. Helmintofauna del "Juile" Rhamdia guatemalensis (Pisces: Pimelodidae), del lago de Catemaco, Veracruz. Rev Soc Mex Hist Nat 1992; 43: 25-31.
- [28] Kamo H, Ogino K, Hatsushika ARA. Unique infection of man with *Clinostomum* sp., a small Trematoda causing acute laryngitis. *Yonago Acta Medica* 1962; **6**: 37–40.
- [29] Hiral H, Ooiso H, Kifune T, Kiyato Y, Sakaguchi Y. Clinostomum complanatum infection in posterior wall of pharynx of human. Jpn J Parasitol 1987; 36(3): 142–144.
- [30] Isobe A, Kinoshita S, Hojo N, Fukushima T, Shiwaku K, Yamane Y. The 12<sup>th</sup> human case of *Clinostomum* sp. infection. *Jpn J Parasitol* 1994; 43(3): 193–198.
- [31] Maejima J, Fukumoto S, Tanihata T, Wang HR, Hirai K. The fourth case of human infection with *Clinostomum* sp. (Trematoda: Clinostomidae) in Shimane prefecture. *Jpn J Parasitol* 1996; 45(4): 333-337.
- [32] Kitagawa N, Oda M, Totoki T, Washizaki S, Kifune T. Lidocaine spray used to capture a live *Clinostomum* parasite causing laryngitis. *Am J Otolaryngol* 2003; 24(5): 341–343.